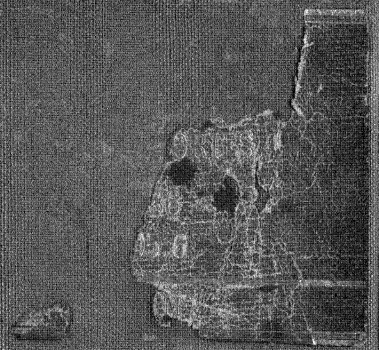

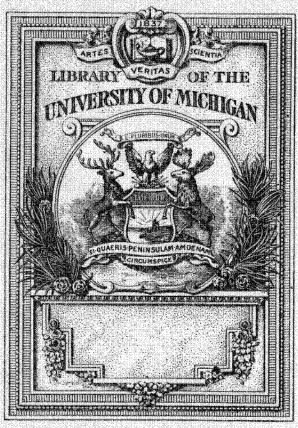

No. 32.—JUNE, 1905

DEPARTMENT OF THE INTERIOR

BUREAU OF GOVERNMENT LABORATORIES

BIOLOGICAL LABORATORY

I. INTESTINAL HEMORRHAGE AS A FATAL COMPLICATION IN AMŒBIC DYSENTERY AND ITS ASSOCIATION WITH LIVER ABSCESS

By RICHARD P. STRONG, M. D.

II. THE ACTION OF VARIOUS CHEMICAL SUBSTANCES UPON CULTURES OF AMŒBÆ

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III. THE PATHOLOGY OF INTESTINAL AMŒBIASIS

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MANILA
BUREAU OF PUBLIC PRINTING
1905

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(Continued on third page of cover).

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LETTER OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, P. I., May 25, 1905.

SIR: I have the honor to transmit herewith and to recommend for publication, "I. Intestinal Hemorrhage as a Fatal Complication in Amœbic Dysentery and Its Association with Liver Abscess;" "II. The Action of Various Chemical Substances upon Cultures of Amœbæ," by Dr. J. B. Thomas, attending physician to the Civil Sanitarium, Benguet; "III. The Pathology of Intestinal Amœbiasis," by Dr. Paul G. Woolley, Director of the Serum Laboratory, and Dr. W. E. Musgrave, Pathologist, Biological Laboratory.

Very respectfully,

RICHARD P. STRONG,
Director Biological Laboratory,
Acting Superintendent Government Laboratories.

Hon. DEAN C. WORCESTER,
Secretary of the Interior, Manila, P. I.

INTESTINAL HEMORRHAGE AS A FATAL COMPLICATION IN AMOEBIC DYSENTERY AND ITS ASSOCIATION WITH LIVER ABSCESS.

By RICHARD P. STRONG, M. D., *Director Biological Laboratory.*

Death may occur in amœbic dysentery from the gravity of the intestinal lesions; from exhaustion in protracted cases; from severe complications, particularly such as peritonitis due to the perforation of an ulcer in the large intestine or appendix or an abscess of the liver or lung; from a terminal infection sometimes entering through the ulcerations in the large bowel; from intercurrent disease, and from severe intestinal hemorrhage. The last is of unusual occurrence and is a particularly rare fatal complication.

While the presence of more or less blood in the stools in this variety of dysentery is in fact a common symptom of the disease, and while at times the discharges consist almost entirely of blood and mucus, it is obviously not to these conditions that I wish to refer in this paper; instead it is to the copious intestinal hemorrhage in which several hundred cubic centimeters of fresh blood are passed—such as one sometimes sees, for example, in typhoid fever and from which patients may succumb—that I wish here to invite attention.

Upon reviewing the literature I find that but little notice has been attracted to this complication. Of the recent text-book articles on the subject Scheube,[1] in his description of gangrenous dysentery, states that occasionally large quantities of pure blood are passed and even death may result from bleeding. Manson[2] calls attention to the fact that whenever, in gangrenous dysentery, sloughs separate, hemorrhage is always possible and that sudden collapse may occur from this cause even in otherwise mild cases. Sodre[3]

[1] *Die Krankheiten der Warmen Länder.*

[2] *Manual of Tropical Diseases.*

[3] *Twentieth Century Practice of Medicine,* Vol. XVI.

mentions that in some cases of acute and chronic dysentery an abundant hemorrhage of the intestine may be observed. When it supervenes in an individual already weakened by former losses or by many days of disease, death may result from it, the patient dying in collapse. None of these authors, however, refer particularly to hemorrhages in amœbic dysentery. Kruse and Pasquale[1] in their extensive monograph do not mention severe hemorrhage in amœbic enteritis, and Harris,[2] in a summary of his own thirty-five cases of the amœbic variety and of seventy-eight others collected by him in the United States, also does not refer to this complication. Osler,[3] however, calls attention to it in acute amœbic dysentery, and states that, of the cases admitted to his wards during the past twelve years, there were seven in which hemorrhage occurred from the bowel. The only direct reference I have been able to find in the literature of amœbic dysentery in which the patient appeared to succumb from the loss of blood is one reported by Loeffler.[4] In this case only 125 cubic centimeters of clotted blood were passed from the rectum. The author states that here a diphtheritic inflammation of the intestine was added to the amœbic infection. It was the only instance of this nature observed by Loeffler.

The following cases of amœbic dysentery are the only ones which have come under my notice which have succumbed to the hemorrhage and therefore they seem worthy of report:

CASE NO. 1. AMŒBIC DYSENTERY; LIVER ABSCESS; SEVERE MULTIPLE INTESTINAL HEMORRHAGES; DEATH; AUTOPSY.

The patient, a well-to-do merchant, aged 36 years, had resided in Manila for the past two years. On February 4, 1902, he consulted the author, complaining of a dysentery of several weeks' duration. An examination of the stools showed the disease to be of the amœbic variety, the fæces containing considerable blood and mucus and many actively motile amœbæ, some inclosing red blood cells. He was advised to enter the hospital for treatment, which he did. On admission the subcutaneous fat was everywhere very abundant. The tongue was lightly coated and the conjunctivæ of good color. The examination of the heart and lungs revealed

[1] *Zeitsch. für Hygiene*, 1894.
[2] *American Journal Med. Sciences*, 1898.
[3] *Practice of Medicine.*
[4] *Allbutt's System of Medicine*, Vol. II.

nothing abnormal. The spleen was not palpable and the liver not enlarged. The abdomen was not distended and there was no pain on pressure. The temperature registered 99° and the pulse 72. The examination of the urine showed nothing pathological. The patient was placed upon liquid diet, and given one-half ounce Rochelle salt. Local treatment consisting of high enemata of quinine solution (1–5,000) was then begun and administered daily, the strength of the solution being gradually increased to 1–500 and the amount of fluid employed from 1 to 2 liters. Under such treatment, and with occasional saline purges, he gradually improved. The tenesmus and irritability of the large bowel gradually decreased and the blood and mucus almost entirely disappeared from the fæces, so that after three weeks' treatment the bowel movements became reduced to one or two per day and the patient was up and about, though still under treatment. The case seemed to be progressing favorably.

However, on February 25 the temperature, which had not been above 99.5°, rose to 103°, and the patient complained of headache and some pain in the chest. There was one bowel movement on this date. On February 26 the temperature remained in the neighborhood of 102°, but the patient complained of no pain. The bowels did not move for twenty-four hours. On the morning of the 27th a blood examination of a fresh smear revealed some increase of the white blood cells and a blood count showed 25,000 leucocytes. He was given one-half ounce of Rochelle salt, and an examination of the fluid stool passed shortly after revealed no blood. On microscopical examination a fair number of amœbæ and some epithelial cells and leucocytes were present. On February 28 the morning temperature registered 102°. The conjunctivæ were slightly tinged with yellow. There was still complaint of some pain in the right side of the chest, but most of the pain was referred to the right inguinal region. The edge of the liver was not palpable. A blood count showed 28,000 leucocytes. A diagnosis of liver abscess was made and the patient was transferred to the surgical side of the hospital. The bowels moved but once on this date. On March 1 the temperature ranged between 102° and 103° and on March 2 it touched 104.2°.

On March 3 he was operated upon. An incision was first made over the right hypochondriac region just below the costal margin and the lower portion of the right lobe of the liver exposed. An attempt

was then made to locate the abscess through aspiration of the various portions of the liver with a long needle. This, however, failed, and the liver was stitched to the abdominal wall and the patient returned to the ward.

On March 2 and 3 there were no bowel movements, but on the 4th the bowels moved four times during the day. The stools were thin and yellow, but only the first contained a little blood. On March 5 there were three bowel movements at night. These were yellow, formed, and contained no blood. It should be mentioned that the local treatment with enemata had been discontinued since February 26. The fever still continued. On March 6 there were six bowel movements of greenish-yellow color containing some milk curds and other undigested food.

The patient was seen again by the author on March 7. He then complained of pain in the region of the operation wound. While asleep there was considerable muttering and marked twitching of the hands. The temperature was 103.4°, the pulse 110. There was very slight jaundice of the conjunctivæ. The abdomen was slightly distended. A blood count showed 18,000 leucocytes. There were three bowel movements on this date, one containing a little blood and mucus. A microscopical examination showed many amœbæ, some inclosing red blood cells. A diagnosis of typhoid fever was suggested by one of the staff in consultation and was particularly urged, as the abscess had not been located, but arguing against such a diagnosis were the facts that the spleen was not palpable and there were no rose spots. Moreover the serum failed in the afternoon of this day to give an agglutinative reaction with *Bacillus typhosus*. It was suggested that the local treatment with quinine enemata be resumed and that another attempt be made to locate the abscess. Accordingly aspiration was again performed by the surgical staff through the abdominal wound, but still unsuccessfully. On March 8 there were two bowel movements after the enema of quinine solution, and on the 9th four. The movements were dark and thin, but macroscopically contained no blood. The temperature ranged between 102° and 103.6°.

On March 10, at 2.45 a. m., a large hemorrhage of about 500 cubic centimeters of fresh-looking blood and containing four or five large clots were passed from the rectum. The pulse shortly after counted 140. The temperature was unfortunately not taken until two hours later, when it registered 102.6°. The pulse then

counted 134. The patient complained of great thirst, but apparently suffered no pain. On the morning of March 10 he was again seen. The subsultus of the hands was marked and there was some muttering delirium. The pulse was 120, of high tension but not dicrotic. His condition at this time suggested typhoid fever—a diagnosis, in fact, adhered to by one of the hospital staff—yet upon a careful analysis of the symptoms of the case the diagnosis of typhoid hardly seemed justifiable, and the serum again gave no Widal reaction. At 11.30 a. m. of this day a second intestinal hemorrhage occurred, about 300 cubic centimeters of dark blood being passed. On the following day the intestinal symptoms seemed a little improved, but the leucocytosis and fever continued. On March 12, at 3.30 p. m., 400 cubic centimeters of fresh blood was passed from the rectum. The temperature dropped to 101° and the pulse became very weak and counted 140. An hour later another hemorrhage of about 200 cubic centimeters occurred. At 6.30 p. m. a large amount of clotted blood was passed. The patient complained of great exhaustion and weakness. At 8 p. m. another large hemorrhage occurred and at midnight and again at 12.30 a. m. smaller hemorrhages were passed. The pulse gradually weakened and increased in rapidity. Finally it no longer could be counted. The patient became very delirious and died during the night. Shortly before death there was a dark-brown watery stool.

At autopsy a large abscess measuring 12 centimeters in diameter was found in the right lobe of the liver situated superiorly and near the posterior surface. The liver was not enlarged. The gall bladder and ducts were normal. The spleen also showed no pathological change. The walls of the large intestine were not particularly thickened and there was no excessive œdema of the submucous coat. In the ascending, transverse, and upper portions of the descending colon there were about fifty or sixty ulcers scattered here and there, generally with even margins and with clean bases. Their edges were very slightly undermined. They measured from about 3 to 12 millimeters in diameter and about $1\frac{1}{2}$ to 2 millimeters in depth. Approximately 5 centimeters below the cæcum was an ulcer filled with a lightly adherent clot. On removal of the clot a freshly thrombosed vessel could be detected. At the edge of the ulcer the vessel was injected and could be traced with the naked eye for about 1 centimeter in the submucosa. There was no

diphtheritis in the large bowel. The mucous membrane between the ulcers was pale in color. The ulcers were clean, and nothing in their appearance suggested a fatal issue for the disease other than that of the one containing the blood clot. The ileum appeared normal. There were no evidences of typhoid fever.

The second case to which I wish to refer was seen in consultation with Dr. Otto Bartels, of Manila.

CASE NO 2. AMŒBIC DYSENTERY; LIVER ABSCESS; MULTIPLE SEVERE INTESTINAL HEMORRHAGE; DEATH; AUTOPSY.

The patient gave a history of having had several attacks of diarrhea during the past year, but had not noticed any blood in his stools. Since his entrance to the hospital, one week before, he had been complaining particularly of headache and restlessness. At times he had slight delirium. There was some constipation during this period, and purgatives and enemata were prescribed for him upon several occasions. Amœbæ were present in his stools. His temperature for four days previous to the time I first saw him, April 8, had varied between 99.4° and 102.6°. There was no distinct jaundice. Owing to the pain in the right hypochondriac region, to the fever, and leucocytosis of 23,000, a diagnosis of liver abscess was made and an operation advised. The patient, however, would not consent to an operation.

On April 8 there was one bowel movement, but none on the following day. On the 10th, 11th, and 12th the bowels moved once each day. The stools contained some mucus, and on microscopical examination, in addition to a few red blood cells, a number of motile amœbæ were observed. On April 11 hiccough appeared and persisted for several hours. At 5 p. m. April 13 a hemorrhage occurred from the bowel of about 200 cubic centimeters of fresh blood. The pulse remained good, but the temperature fell from 101.5° to 98° two hours later. Early on the following morning the patient complained of pain in the abdomen, and shortly afterwards a large amount of fresh and partially clotted blood was expelled from the intestine. Two hours later there occurred another hemorrhage of about 400 cubic centimeters of bright red blood. The pulse became considerably weaker after the second hemorrhage and the temperature fell nearly four degrees in three hours. The patient suffered from nausea and vomiting at intervals through the day and gradually became weaker. On the following day the pulse became very feeble. The vomiting continued until within a few

hours of his death, which occurred on the following morning. There were no more hemorrhages or bowel movements.

At autopsy there was a large abscess measuring about 14 centimeters in diameter situated in the right lobe of the liver. The left lobe contained a small abscess measuring about 7 centimeters in diameter. The liver tissue was very fatty. The gall bladder and ducts were normal. The large intestine contained many shallow ulcerations, some of which were in the healing stage. The large bowel contained some dark clotted blood. After a careful search I was unable to locate any specific point from which the hemorrhage had occurred. Scrapings from the intestinal ulcers and from the walls of the abscess showed many motile amœbæ, some containing red blood cells.

The question suggests itself of why severe intestinal hemorrhage is not of more frequent occurrence in amœbic dysentery, particularly when one considers the extensive lesions of the submucosa which are present in most of the advanced cases. However, the additional points in the pathology of the infection which would tend to prevent hemorrhage must be recalled, viz, the thrombosed condition of the blood vessels in the zone of infiltration and œdema which surrounds the ulcers, the infiltration of the walls of the arteries, and the more or less marked evidence of endarteritis as the progress is rapid or slow. In chronic cases one may see at times the lumina of the arteries entirely occluded by this process.

On the other hand, the frequent occurrence of smaller amounts of blood in the stools may be explained from the fact that the walls of the veins are early infiltrated with round cells, followed by softening and complete disorganization; also from the fact that amœbæ may penetrate the wall of a vein. However, thrombosis of the veins is not infrequent.

As a rule the blood in the stools in amœbic dysentery probably arises not from one but from many ulcers about which the capillaries are usually considerably distended, frequently forming a network at the bases and margins of healing ulcers. At post-mortem, when one removes the upper layer of the mucosa in the vicinity of an ulcer, one frequently finds small hemorrhages in the upper portion of the submucosa. When the overlying mucosa becomes necrosed and sloughs, the blood from these vessels finds its way into the lumen of the intestine and appears later in the stools. However, in the cases which we have mentioned above the hemorrhage probably arose from a single ulcer involving a blood vessel.

Since the foregoing observations were recorded to the Manila Medical Society in 1902, F. Haasler,[1] in the same year, in an article treating of the complications of amœbic dysentery and reviewing 600 cases of the disease occurring in China, mentions three of severe intestinal bleeding, in two of which the hemorrhage was considered the cause of death. In one of the cases about 4 liters of blood was passed and the author was able to find at necropsy a thrombosed vessel from which the bleeding occurred. A most interesting fact in connection with these cases and one emphasized by the author is that, in both of the fatal instances in which death was due to the hemorrhage, liver abscess coexisted.

Woodward[2] in 1879 also reported two cases of dysentery in which profuse hemorrhage occurred and in which large liver abcesses also existed. In the first instance death resulted immediately from the hemorrhage. Though the cases were not diagnosed as those of amœbic dysentery, there can be little doubt from the histories and autopsies that they were indeed instances of this variety of the disease.

During the past two years I have encountered two more fatal cases of amœbic dysentery with severe multiple hemorrhage in both of which large liver abscess was present. In the last one the time of coagulation of the blood was not complete until nine minutes. There was no marked jaundice present. These cases may here be briefly recorded:

CASE NO. 3. CHRONIC AMŒBIC DYSENTERY; MULTIPLE LIVER ABSCESS; SEVERE INTESTINAL HEMORRHAGES; DEATH; AUTOPSY.

The patient, age 27 years, was first seen in October, 1903. At this time his general physical condition was fair, but he was already suffering with a well-advanced case of amœbic dysentery of about two months' duration. The stools were numerous and contained large amounts of blood-stained mucus. He was placed upon local treatment of high quinine enemata and pursued this treatment daily for nearly four months. During this time his general condition gradually improved. On several occasions, for one or two weeks at a time, the stools became fairly normal, one or two per day, and contained no amœbæ and no mucus or blood; but notwithstanding the fact that the local treatment was continued, the disease always broke out afresh and amœbæ and mucus and blood reappeared in the stools.

[1] *Deutsche Medicin Wochenschr., 1902.*

[2] *Medical and Surgical History of War of the Rebellion,* II Med. Vol., pp. 164 and 209.

However, in January he felt sufficiently improved to leave Manila for Japan, where he remained for about three months. During some of this time he neglected treatment entirely. For the first month he reported himself to be fairly well, but shortly afterwards an acute exacerbation of the dysenteric symptoms appeared and he was compelled to enter a hospital. As soon as his condition temporarily improved he returned to Manila. He was seen again by the writer on April 9; at this time he was considerably emaciated and his face was drawn. He complained of an aching sensation in the right shoulder. The liver was distinctly palpable for several fingers' breadth below the costal margin. The temperature registered 100° and there was a leucocytosis of 15,000. His pulse counted 112. The question of an operation for liver abscess was considered, but was not immediately urged. Owing to the chronic character of the dysentery and the general condition of the patient, it was decided that an attempt should first be made to ameliorate the dysenteric symptoms. Therefore he was again placed upon quinine enemata with occasional doses of Dover's powder and was given, in addition, stimulants with the hope that in a few days his condition might so improve as to warrant an operation. His diet consisted only of liquids. The temperature ranged for the next two days between 100.2° 103.2°. The bowel movements numbered three or four per day and usually contained considerable mucus. On April 12, at 9 a. m., his daily quinine enema was administered. During the day there were two bowel movements, the last at 3 p. m. At 7 p. m., a large intestinal hemorrhage occurred, nearly a pint of fresh blood being passed. At 9 a. m. a second hemorrhage, smaller in amount but of the same character, occurred. At 12.30 a. m. a large amount of dark blood was passed. Morphia was administered hypodermically, and later, ergot. Finally a hot enema of tannic acid was given, but apparently no favorable results were obtained. Between 12.30 and 8 o'clock the next morning there were five small hemorrhages. At the latter hour the temperature registered 99° and the pulse 138. The patient gradually sank. There were no more large hemorrhages from this time up to that of his death, which occurred at 7 a. m. the following morning, but the movements which occurred and were passed into a bedpan, consisted almost entirely of clotted blood.

At autopsy the large intestine showed extensive ulcerations throughout. The ulcers were as a rule shallow, usually undermined, and with smooth or slightly uneven reddened margins. In the cæcum deeper ulcerations were present and between these lesions portions of the mucosa were covered with pseudomembrane. Some of the ulcers in the cæcum were gangrenous. The contents of the large bowel consisted of dark-reddish masses of fluid and partially clotted blood, together with some mucus.

The lower end of the ileum for about 15 centimeters above the valve also showed ulceration. No distinct point from which the hemorrhages arose could be detected anywhere in the entire intestine.

There were six abscesses of the liver situated in both the right and left lobes and measuring from 5 to 10 centimeters in diameter. A number of the hepatic veins contained thrombi.

CASE NO. 4. AMŒBIC DYSENTERY; LIVER ABSCESS; SEVERE INTESTINAL HEMORRHAGE; DEATH; AUTOPSY.

The patient, a Spanish sailor, was first seen after an alcoholic debauch. At this time he was dull and stupid. He complained of acute dysentery. A companion stated that he had been bleeding extensively from the rectum during the previous day. At the time of my visit his temperature registered 99°, and the pulse counted 114. No distinct history of previous dysentery could be obtained. The patient refused to enter a hospital. A portion of a bowel movement, consisting of reddish-brown masses of blood and mucus, was secured, and a microscopical examination showed numerous amœbæ and red blood cells, and considerably altered blood pigment. Later in the day a blood count showed 9,000 leucocytes per cubic millimeter. The coagulability of the blood was tested and found to be complete only after nine minutes. The liver dullness was distinctly increased upward in the right axillary line above the fifth rib. The patient complained of slight pains below the right axillary region. The conjunctivæ were slightly jaundiced. Morphia, calcium chloride, and absolute rest were prescribed. The patient was seen again on the evening of the same day. At this time his pulse counted in the neighborhood of 150 and was weak and thready. The extremities were cold. He was already unconscious. His companion stated that he had passed three or four large hemorrhages from the bowels during the day. The sheet upon which he was lying partially disclosed this fact, being in places soaked with fresh blood. He gradually sank and died during the night. A complete autopsy could not be performed, but an incision was made over the right hypochondriac region, the liver drawn down, and the diagnosis of abscess in the right lobe confirmed.

On recalling the infrequency of fatal hemorrhage in amœbic dysentery it seemed to me that some reason other than the anatomical situation of the ulcer might exist in the eight cases referred to above and which might account for the persistence of the bleeding and for their unfavorable outcome. Since in all of the cases large liver abscess co-existed, the connection between intestinal hemorrhage and the hepatic condition has suggested itself very strongly to me. The idea that the destruction of such large amounts of liver tissue may sometimes bring about serious functional disturbance in this organ and lead to a condition which predisposes to hemorrhage must certainly be considered. James Finlayson,[1] as long ago as 1873, in discussing a case of liver abscess in which intestinal hemorrhage had occurred, argued that hepatic abscesses by interfering mechanically with the portal circulation may produce congestion of the mucous membrane of the colon and thus favor

[1] *Glasgow Medical Journal*, Feb., 1873, p. 171.

the development of hemorrhage. The relation between hemorrhage and various other diseases of the liver, such as acute atrophy, syphilis, cancer, and affections of the biliary passages, particularly when jaundice is present, is well known. However, it is true that in typhoid fever severe and fatal intestinal hemorrhage may occur independently of any extensive lesion of the liver, though it has even been claimed that when such a result takes place it depends chiefly upon a diminished coagulability of the blood[1] or to special bacterial activity.[2] Therefore, while it obviously is probable that more extensive observations will show that fatal intestinal hemorrhage in amœbic dysentery may occur independently of liver abscess, the cases to which I have referred would seem to point out that at least when hemorrhage occurs in cases complicated with such hepatic disease, it is likely to be very severe and that the bleeding is likely to recur.

It is also possible that the occurrence of multiple intestinal hemorrhages in amœbic dysentery may occasionally be of some importance in the diagnosis of liver abscess. In my last case as noted above there was no fever and no leucocytosis, and although the liver was slightly enlarged and abscess was suspected, I did not feel by any means certain of such a diagnosis. However, when the intestinal hemorrhages appeared, reasoning from my knowledge of the conditions in the other five cases, I felt confident of the existence of hepatic abscess, a diagnosis which, as already mentioned, was confirmed at autopsy. In this connection I was recently much interested to find in Woodward's article on dysentery in the *Medical History of the War of the Rebellion,* 1879, the statement that "hemorrhage from the bowels is another occasional symptom of liver abscess and sometimes is the immediate cause of death." This statement seems to have received no attention in the literature on amœbic dysentery.

We are about to undertake by Wright's method a study of the coagulability of the blood in our cases of amœbic dysentery, for the purpose of ascertaining if any changes occur either during the course of the uncomplicated disease or in those cases in which liver abscess or hemorrhage develop.

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[1] Wright and Knapp, *Lancet,* 1902, Vol. II, pp. 16, 1533.

[2] Nicholls and Learmonth, *ibid.,* 1901, Vol. I, p. 305.

THE ACTION OF VARIOUS CHEMICAL SUBSTANCES UPON CULTURES OF AMOEBAE.

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In considering the subject of the local treatment of amœbic dysentery one encounters a long list of drugs recommended by individual experimentors and clinicians, but the one proposed in some form or other by the large majority of the medical profession is quinine. It is employed in solutions varying in strength from 1–5,000 to 1–3,000, from one-half to 1 liter, two or three times a day, as recommended by Councilman and Lafleur in their classical monograph published in 1891, all the way up to the excessive concentration of 1–100 advised by J. H. Ford in a recent number of the *Journal of Tropical Medicine.* Osler recommends a warm solution of quinine 1–5,000, 1–2,000, 1–1,000, and states that it has been used with great benefit in the wards of the Johns Hopkins Hospital.

Councilman and Lafleur summarize their experience with quinine enemas as follows:

> Quinine injections do destroy amœbæ in the bowel, but it is questionable if they reach the amœbæ in the tissues. Such treatment is serviceable in early cases and those where the rectum, sigmoid flexure, and descending colon are the limit of the disease.

Among the drugs less commonly advised for the local treatment of amœbic dysentery we find the following: Bichloride of mercury 1–5,000 to 1–3,000; nitrate of silver 1–500; dilute nitric acid (by H. A. Lafleur in *Allbutt's System of Medicine*).

Cold water enemas (by Tuttle).

Potassium permanganate 1–1,000 to 1–2,000; eucalyptol 1–1,000; sodium bicarbonate 1–100 (by J. H. Ford).

Tannin 1–200; ichthyol 1–250, in combination with salts of bismuth (by Hemmeter).

However, it will probably be admitted by those who treat large numbers of amœbic dysentery cases that the ideal substance for local treatment has not yet been discovered. Weak solutions of quinine fail to destroy the amœbæ, which frequntly persist in the intestine

in spite of two or three large injections daily; strong solutions are often too irritating to be practical or, in the case of susceptible subjects, may reduce the patient to a state of chronic cinchonism by the absorption of the drug from the bowel. This last condition is more apt to occur where the practice prevails of giving several concentrated injections a day.

The stronger antiseptics are likely to be irritating or dangerous on account of their toxic properties.

The perfection of the technique of growing the amœbæ in pure strains in symbiosis with a single variety of bacteria attained by Musgrave and Clegg has made it possible to observe with a considerable degree of accuracy the action of chemical substances on the amœbæ, and it is with the results of a series of such experiments conducted by myself in the Biological laboratory of the Bureau of Government Laboratories during December, 1904, and January, 1905, that this report is concerned.

The standard amœba used in these tests is described as follows by Musgrave and Clegg in *Bulletin No. 18, Biological Laboratory,* of the Bureau of Government Laboratories, published in October, 1904:

Amœba 11524 was isolated from a dysentery stool. The patient, an American nurse, had been suffering with intestinal amœbiasis (amœbic dysentery) for about one year and amœbae had repeatedly been found in her stools during that time. * * * The course of the disease was a usual one, with very chronic tendencies and with frequent, and sometimes quite severe, exacerbations. Our first cultures were made during such an exacerbation and at a time when there could be no reasonable doubt as to the correctness of the diagnosis. * * * Growth was found to be very satisfactory for a long time on a medium composed of 2 per cent agar and one-half per cent beef extract (1 per cent alkaline), the development decreasing only when a marked diminution in the number of bacteria, which is usual with this medium, occurred. Microscopically this protozoön as obtained from culture is indistinguishable from those seen in the stools of the patient, and it is a true dysenteric amœba. Its measurements in the round stage in the stool were 25 to 35 μ, and those in the cultures generally correspond with these figures, but they varied greatly, owing no doubt to environment and the phase of the life cycle at the time of the examination. In our collection there now are cultures of this amœba which were started from a single parasite. They are in pure culture with four different bacteria. * * * *B. coli, Spr. cholerœ* and two different pigment-producing saprophytes. The protozoa grow well with all these organisms, and, by methods already given, have been changed from one to the other, and vice versa.

In one instance, dysentery in man followed the ingestion of three gelatin

capsules filled with scrapings from the surface of cultures of this amœba in symbosis with a harmless bacterium. Dysentery has also been produced in monkeys by similar cultures as well as by others where the bacterium in symbiosis was a pathogenic one.

This amœba in symbiosis with the cholera spirillum was used in the experiments to be described. The culture medium employed was the special agar one recommended by Musgrave and Clegg and described in the above quotation. The amœbæ, in the encysted state, are much more resistant to the action of drugs by virtue of their impervious shells. In order as far as possible to eliminate these encysted forms from the experiments, I made many observations on cultures of the amœbæ at varying periods of their growth, and determined that when they are cultivated on the special agar medium at room temperature in Manila, the amœbæ reach their maximum activity in about forty-eight hours, by which time practically all were free and motile. In a few hours more, many have begun to encyst and by the end of seventy-two hours a large proportion of them have completely done so. Consequently, after the first few preliminary experiments, the forty-eight-hour cultures were used as a matter of routine.

The first series of experiments was made by pouring the solutions to be tested over the surface of forty-eight-hour slant cultures of Amœba No. 11524, and at the end of ten, twenty, thirty, or sixty minutes pouring off the solution, washing the surface lightly with sterile water to remove all traces of the antiseptic, and then making transplants from the surface to fresh media. Control transplants were made from every tube containing the amœba cultures, before adding the solution to be tested.

The following results were obtained by the above-described method:

TABLE A.

Antiseptic.	Dilution.	Antiseptic applied.	Growth of amœbæ after exposure to antiseptic.	Growth of symbiotic bacteria.
		Minutes.		
Benzoyl acetyl peroxide (acetozone)[1]	1-1,000	10	None	No.
Do	1-1,000	30	None	No.
Do	1-1,000	60	None	No.
Do	1-500	30	None	No.
Do	1-500	60	None	No.

[1] The solutions of benzoyl acetyl peroxide and of acid succinic peroxide were so prepared as to contain equal amounts of *active oxygen*. In order to obtain solutions of 1-1,000 based on active oxygen there must be dissolved of pure crystals of benzoyl acetyl peroxide 1.10 grams and of pure acid succinic peroxide 1 gram in 1 liter of water.

TABLE A—Continued.

Antiseptic.	Dilution.	Antiseptic applied.	Growth of amœbæ after exposure to antiseptic.	Growth of symbiotic bacteria.
		Minutes.		
Acid succinic peroxide (alphozone)	1-1,000	10	Very few	No.
Do	1-1,000	30	do	No.
Do	1-1,000	60	do	No.
Do	1-500	30	do	No.
Do	1-500	60	do	No.
Acid, tannic	1-100	10	Very many	Yes.
Do	1-100	20	do	Yes.
Do	1-100	30	do	Yes.
Copper sulphate	1-1,000	10	do	Yes.
Do	1-1,000	20	do	Yes.
Do	1-1,000	30	Many	Yes.
Do	1-2,000	20	Very many	Yes.
Do	1-2,000	30	do	Yes.
Ichthyol	1-250	10	do	Yes.
Do	1-250	20	Many	Yes.
Do	1-250	30	do	Yes.
Oil of cassia	1-5,000	10	Very many	Yes.
Do	1-5,000	20	Many	Yes.
Do	1-5,000	30	do	Yes.
Do	1-10,000	30	do	Yes.
Potassium permanganate	1-2,000	30	do	?
Quassia infusion	W. S. P.	30	Very many	?
Quinine sulphate	1-500	20	do	Yes.
Do	1-500	30	Many	Yes.
Do	1-250	10	Very many	Yes.
Do	1-250	20	Few	Yes.
Do	1-250	30	do	Yes.
Silver nitrate	1-500	30	do	Very few.
Thymol	1-5,000	10	Many	Yes.
Do	1-5,000	20		Yes.
Do	1-5,000	30		Yes.
Do	1-10,000	20	Many	Yes.
Do	1-10,000	30	do	Yes.

It was thought that by treating the amœbæ in surface growth as described in the foregoing table, where the lower strata are protected by the upper ones of amœbæ and bacteria, the conditions encountered would be fairly analogous to those existing in the intestines, where the amœbæ are protected by mucus and fecal matter. Cases in which the amœbæ have burrowed beneath the surface epithelium will not be taken into consideration in this connection, as no form of local treatment would probably avail against such conditions.

The foregoing table is therefore of interest as suggesting the relative action of several chemicals on artificially grown amœbæ under conditions unfavorable to the complete effect of the antiseptic substances and therefore somewhat like the conditions encountered in the practical use of the chemicals in treating the disease locally in the intestine itself.

It will be noted that quinine 1–500 had but a moderate effect

on the amœbæ in thirty minutes and 1–250 a decided one in twenty minutes.

Nitrate of silver, thymol, benzoyl acetyl peroxide, and acid succinic peroxide in moderate strength exercised a marked effect on the amœbæ, whereas sulphate of copper, permanganate of potassium, tannic acid, infusion of quassia, ichthyol, and oil of cassia had but a slight one.

In order to determine more accurately the effect of these and other substances on the unprotected amœba, another series of tests was undertaken on the amœbæ suspended in fluid, and in order to determine whether the deleterious action of each substance was due to a specific action on the amœbæ or to the destruction or attenuation of its symbiotic bacteria, synchronous transplants were made from the treated culture to sterile agar plates and to others previously inoculated with the cholera spirillum.

Uniform suspensions of the amœbæ were made by pouring 4 cubic centimeters of distilled, sterile water over the surface of a forty-eight-hour slant agar culture of the amœba and cholera spirillum, scraping off the surface growth, mixing with the water by means of a platinum wire, and pouring the resultant suspension into a sterile test tube. Four cubic centimeters (in double strength) of the antiseptic solution to be tested were then added to the 4 cubic centimeters of suspension of amœbæ, thus making a fairly uniform solution of 8 cubic centimeters of liquid to one forty-eight-hour slant culture, the mixture containing a definite amount of the chemical to be tested. The suspension was next thoroughly shaken and allowed to stand for the desired lengths of time, and then transfers of one loop of the suspension were made to Petri dishes containing the special agar medium, and allowed to develop for forty-eight hours. At the end of such time the plates were carefully examined for amœbæ and bacteria. As stated before, similar transplants were made at the same time to plates previously inoculated with the cholera spirillum. To minimize the concentration of the small quantity of antiseptic carried over with the loop of suspension, the droplet was spread over a circular area about one-half inch in diameter in the center of the agar plates.

The following table epitomizes the results of such experiments as were carried out with a maximum degree of uniformity and accuracy. Many more tests besides those tabulated were made with each substance, substantiating in the main the results set forth in

the table, but it has not been thought necessary to include them in this report. By always using the same medium, the same strain of amœbæ and symbiotic bacteria, checking every culture and every transplant with controls, using cultures of uniform age (forty-eight hours), cultivating transplants for the same length of time (forty-eight hours), and making the amœba suspensions of uniform concentration (8 cubic centimeters water to one slant culture forty-eight hours old), the relative results are probably as accurate as possible under the circumstances:

TABLE B.

Antiseptic.	Dilution.	Antiseptic applied.	Growth of amœbæ after exposure to antiseptic.	Growth of amœbæ on plates inoculated with cholera.	Growth of symbiotic bacteria.	Growth of control.
		Min.				
Benzoyl acetyl peroxide[1]						
Acid, boric	1-50	15	Rich	Rich	Yes.	Yes.
Do	1-50	30	do	do	Yes.	Yes.
Do	1-50	60	Fair	Fair	Yes.	Yes.
Do	1-25	60	do	do	Yes.	Yes.
Do	1-25	(2)	do	do	Yes.	Yes.
Acid, tannic	1-100	15	Very slight[3]	do	No.	Yes.
Do	1-100	30	do	do	No.	Yes.
Acid, succinic peroxide	1-1,000	15	do	Rich	No.	Yes.
Do	1-1,000	30	do	Fair	No.	Yes.
Do	1-1,000	60	None	Very slight	No.	Yes.
Do	1-2,000	30	Slight	Fair	Yes.	Yes.
Argyrol	1-100	15	Very slight	do	No.	Yes.
Do	1-100	30	do	do	No.	Yes.
Do	1-100	60	None	do	No.	Yes.
Do	1-500	15	Very slight	do	No.	Yes.
Do	1-500	30	do		No.	Yes.
Copper sulphate	1-1,000	30	None	Rich	No.	Yes.
Do	1-1,000	60	do	Very slight	No.	Yes.
Do	1-1,000	(2)	do	do	No.	Yes.
Do	1-2,000	30	Slight	Slight	Yes.	Yes.
Do	1-2,000	60	None	Very slight	No.	Yes.
Do	1-10,000	60	Fair		Yes.	Yes.
Eucalyptol (emulsion)	1-2,000	60	do		Yes.	Yes.
Ichthyol	1-500	60	Slight		Yes.	Yes.
Oil of cassia (emulsion)	1-2,000	30	Fair		Yes.	Yes.
Do	1-2,000	60	Slight		Yes.	Yes.
Protargol	1-500	60	Very slight		No.	Yes.
Do	1-100	30	do	Very slight	No.	Yes.
Do	1-100	60	do	do	No.	Yes.
Potassium permanganate	1-2,000	15	None	None	Yes.	Yes.
Do	1-2,000	30	do	do	No.	Yes.
Do	1-4,000	15	Fair	Fair	Yes.	Yes.
Do	1-4,000	30	Very slight	do	Yes.	Yes.

[1] No experiments were performed with this chemical to obtain comparative results on this table, because it had already been proven by other experiments performed in this laboratory that amœbæ were always killed by benzoyl acetyl peroxide in the usual dilutions.

[2] Two hours.

[3] It is not meant to imply either in this table or in the previous one that where a "very slight" growth of amœbæ on the sterile plate is recorded and no growth of the symbiotic bacteria, that a pure cultivation of the amœbæ without bacteria, has been obtained. A very few bacteria may still be present (perhaps adherent to the protozoa) which are sufficient to nourish the amœbæ and cause a moderate reproduction of them. In transplanting amœbæ another source of error may arise, in regard to their reproduction, where the growth of amœbæ is "very slight," unless the number inoculated on the plate has been counted.—R. P. S.

TABLE B—Continued.

Antiseptic.	Dilution.	Antiseptic applied.	Growth of amœbæ after exposure to antiseptic.	Growth of amœbæ on plates inoculated with cholera.	Growth of symbiotic bacteria.	Growth of control.
		Min.				
Quassia infusion	W. S. P.	60	Very rich	Rich	Yes.	Yes.
Quinine sulphate	1–500	30	None		No.	Yes.
Do[1]	1–1,000	15	Fair	Fair	Yes.	Yes.
Do[1]	1–1,000	30	Very slight	Very slight	No.	Yes.
Do[2]	1–1,000	15	do	Rich	Yes.	Yes.
Do[2]	1–1,000	30	do	do	No.	Yes.
Quinine bisulphate[2]	1–1,000	15	None	Slight	Yes.	Yes.
Do[2]	1–1,000	30	do	Fair	No.	Yes.
Silver nitrate	1–500	30	do		No.	Yes.
Do	1–500	60	do		No.	Yes.
Do	1–2,000	15	Very slight	Very slight	No.	Yes.
Do	1–2,000	30	do	do	No.	Yes.
Thymol	1–5,000	30	Fair	Slight	Yes.	Yes.
Do	1–5,000	60	None	None	Yes.	Yes.
Do	1–2,500	15	do	do	Yes.	Yes.
Do	1–2,500	60	do	do	Yes.	Yes.

[1] HCl 1–5,000. [2] HCl 1–2,500.

In examining the foregoing table the following results will be noted:

Boric-acid solution as strong as 1–25 had practically no effect on the amœba or the cholera spirillum after an exposure of two hours.

Tannic acid 1–100 after thirty minutes had a moderate effect on the viability of the amœba apparently by destroying almost all of the cholera spirilla in the culture.

Succinic peroxide acid exercised a marked deterrent effect on the growth of the amœba by destroying most of the spirilla, as was demonstrated by the fact that the transfers to cholera plates always contained a much larger proportion of amœbæ than did the synchronus transfers to sterile agar plates. For benzoyl-acetyl peroxide see preceding table.

Nitrate of silver showed a marked destructive effect both on amœbæ and on bacteria, 1–2,000 applied for fifteen minutes destroying the bacteria and leaving a very slight growth of amœbæ on both sterile and cholera plates; 1–500 destroyed both amœbæ and spirilla in thirty minutes.

Among the colloidal silver salts tested, argyrol as weak as 1–500 applied fifteen minutes exercised an effect similar to that of succinic peroxide acid and protargol; 1–500 applied for one hour left almost no surviving spirilla and very few amœbæ.

Eucalyptol emulsion 1–2,000 (with bicarbonate of soda), ichthyol 1–500, and oil of cassia in emulsion 1–2,000 all permitted a fair growth of amœbæ and cholera spirilla after an hour's application.

Permanganate of potassium 1–4,000 had but slight effect in fifteen minutes and 1–2,000 stopped all growth of amœbæ after an exposure of fifteen minutes, though failing to destroy the spirilla.

Infusion of quassia really seemed to stimulate the growth of the amœbæ, perhaps supplying some nutritive substance from the wood fibers. Quassia was tried because of its former reputation as an injection for *Oxyuris vermicularis,* and upon the supposition that the bitter principle might exert a harmful effect on the amœba.

Sulphate of quinine 1–500 destroyed amœba and spirilla in thirty minutes; 1–1,000 (acidified with HCl 1–5,000) had a slight deterrent effect in fifteen minutes and marked destructive effect in thirty minutes; 1–1,000 (HCl 1–2,500) had the same effect as the solution of half that acidity, except that the growth of amœbæ on the cholera plates was richer in the former. This action was probably due to the accidental transplanting of an unusual number of amœbæ, as the increased acidity would certainly not favor the growth of amœbæ.

Bisulphate of quinine did not differ appreciably in its action from that of the sulphate when tested in solutions of equal strength and acidity.

Thymol 1–2,500 exercised a marked effect on the amœba in fifteen minutes but failed to destroy the spirillum in an hour. A number of other experiments with thymol even diluted as high as 1–5,000 demonstrated this specific effect on the amœba and a failure to destroy the symbiotic cholera spirillum. The specific action of thymol suggests a combination of substances for the local treatment of amœbiasis in which thymol would form the antiamœbic ingredient and one of the silver salts benzoyl acetyl peroxide or succinic peroxide acid the antibacterial ingredient. Theoretically such a combination as this or the alternate use of the solutions suggested should give the best possible results. The possibility of absorbing thymol in toxic amounts in such high dilutions is remote; however, its local effect on the bowel must be tested clinically.

Sulphate of copper 1–1,000 stopped the growth of amœbæ in thirty minutes by destroying the cholera spirilla, and 1–2,000 exercised a similar but weaker action in the same length of time.

In view of the general scientific interest awakened as to the use of sulphate of copper in high dilutions as a purifier of water reservoirs, following G. T. Moore's bulletin [1] on that subject, a special series of experiments was undertaken with high dilutions of sulphate of copper on various suspensions of amœbæ. The importance of the subject in connection with the amœba-infected water supply of Manila is very apparent and was long since recognized by the Board of Health for the Philippine Islands. In compliance with a request from Maj. E. C. Carter, Commissioner of Public Health, I reported the results of my experiments with high dilutions of copper sulphate to Dr. R. P. Strong, the Director of the Biological Laboratory, under date of January 30, 1905. The results are in part as follows: After an application of a solution of cupric sulphate 1–5,000 for one hour, many amœbæ and a few spirilla grew on the transplants; after 1–10,000 for two hours, many amœbæ and a few spirilla grew; after 1–100,000 for forty-eight hours, many amœbæ and a few spirilla grew. At a later date the above test was repeated under practically the same conditions, except that control transplants were made to cholera plates, and both the amœbæ and the spirilla grew equally well after exposure for forty-eight hours to copper solutions 1–100,000 and 1–200,000.

All the tests mentioned thus far were conducted with amœba suspensions of 8 cubic centimeters of fluid to one forty-eight-hour slant agar culture of amœbæ and spirilla. Another set of tests was carried out to compare the effects of the copper solutions on amœbæ suspensions of varying concentration. At the same time controls of the suspensions of amœbæ were made, using precisely the same dilutions as those treated with copper and making transplants from these controls and the suspensions treated with copper, at periods varying from twenty-four to ninety-six hours. It was thus possible to determine by comparison to what extent the destruction or attenuation of amœba suspensions might be due to an unfavorable medium, diminished nourishment, etc. The following were results obtained by treating for ninety-six hours three different concentrations of amœba suspensions with copper sulphate 1–100,000:

Eight-cubic centimeter suspension: Transplants developed rich growth of amœbæ and fair growth of spirilla.

[1] *Bulletin No. 64, Bureau of Plant Industry, U. S. Dept. of Agriculture.*

Sixteen-cubic centimeter suspension: Transplants same as 8-cubic centimeter suspension.

Thirty-two-cubic centimeter suspension: Transplants developed few amœbæ and few spirilla.

The 16-cubic centimeter control suspensions with copper omitted, gave practically the same results as did those containing the copper, except that the cholera spirillum grew rather feebly in those treated with copper but freely in those untreated.

In the 32-cubic centimeter suspensions without copper the growth of amœbæ and spirilla was distinctly better than in those treated with copper. The transplants from the suspensions treated with copper to cholera plates developed a rich growth of amœbæ.

We may fairly make the following deductions from the foregoing experiments: First, that high dilutions of cupric sulphate have practically no effect within ninety-six hours, upon concentrated suspensions of amœbæ (8-cubic centimeter solution to one forty-eight-hour slant agar culture); second, that copper solutions as dilute as 1–100,000 have little, if any, specific effect on the amœbæ, though they inhibit the growth of the cholera spirillum to an appreciable extent after an exposure of ninety-six hours, in dilutions as high as 32 cubic centimeters of copper solution to one forty-eight-hour slant culture, and thus impede somewhat the development of the amœbæ. However, it is very doubtful whether this inhibiting action on the spirilla is of sufficient potency, even in dilutions of 1–100,000, to exercise any practical effect on the development of amœbæ in large bodies of water.

After presenting several tables of experimental data as to the action of "colloidal solutions of copper" on the colon bacillus, the cholera spirillum, and other bacteria, Moore makes the following statement in *Bulletin No. 64* mentioned above:

> It is evident that the amount of surface exposed in any ordinary copper tank would far exceed the amount demanded for the above results, and it is likewise certain that after standing from six to eight hours at room temperature in a clean copper vessel water becomes safe to drink even though it may have contained cholera and typhoid germs. It remains to be seen whether or not the application of these facts to conditions in the Tropics, where cholera is abundant, will be of any value. It would seem that the construction of canteens and other water vessels from copper might serve as an additional safeguard, if not an actual preventive of this disease, and would prove of considerable value where distillation or efficient filtration apparatus is not at hand.

In accordance with the above suggestions suspensions of amœbæ and cholera spirilla of varying degrees of concentration were poured into clean copper crucibles, covered, and allowed to stand at room temperature in Manila for five days. At the termination of nineteen hours and of five days, transplants were made in the usual manner with the following results:

Suspension A (4 cubic centimeters to one slant agar culture) developed a good growth of amœbæ and spirilla from transplants made after standing nineteen hours in the copper crucible, and a fair growth on cholera plates after five days, though none on sterile agar plates.

Suspension B (8 cubic centimeters to one slant agar culture) developed a slight growth of both amœbæ and spirilla on sterile agar plates from transplants made after standing five days, and a rich growth on cholera plates.

Suspension C (12 cubic centimeters to one slant agar culture) gave results identical with those of suspension A.

It would appear from the preceding results that it would be disastrous to rely on the action of copper containers to purify water infected with amœbæ or cholera spirilla and that Moore's claim quoted above can not be substantiated, at least as far as it relates to the organisms used in my experiments.

As previously stated in this report, amœba No. 11524 was chosen as a standard because of its proven pathogenicity and its sturdy resistance to unfavorable conditions. It was taken for granted that any chemical substance which would destroy amœba 11524 would destroy most of the others, and the few experiments I had time to carry out in that connection justified the assumption.

The amœbæ employed in these control experiments were No. 39888, a small intestinal amœba isolated by Dr. Musgrave from a case of intestinal amœbiasis and cultivated in symbiosis with the cholera spirillum, and amœba tap "A," isolated by myself from the Manila water supply, drawing the water from the laboratory tap. The latter amœba was cultivated in pure strain from a single individual, in symbiosis with two or three varieties of water bacteria among which a yellow pigment-forming bacillus predominated almost to the complete exclusion of the others.

These two amœbæ were tested with sulphate of quinine 1–1,000 (HCl 1–5,000), thymol 1–5,000, acid succinic peroxide 1–1,000

and nitrate of silver 1–2,000, for periods of fifteen, thirty, and sixty minutes, with the result that the destructive effect of these chemical substances was decidedly more marked than in the experiments in which Amœba 11524 was the organism employed.

Experiments on encysted cultures of No. 11524, one month old, conducted at the same time as the above and with the same solutions, demonstrated the self-evident fact that encysted amœbæ are much more resistent to chemical action than are the free and active forms.

I regret that time did not permit me to pursue my investigations with a larger variety of chemicals and with other strains of amœbæ in symbiosis with various bacteria. However, it may safely be assumed that the results with thymol solutions would be unaffected by such tests as far as they relate to symbiotic bacteria, and it is improbable that any of the common intestinal bacteria usually found in symbiosis with the amœba would resist the action of the silver salts, benzoyl acetyl or acid succinic peroxide to a sufficient extent to alter the deductions that may be drawn from the above experiments in which cholera spirilla and water bacteria were the only symbiotic organisms employed. It should be borne in mind that the entire series of tests was severe on account of the concentration of the amœba suspensions employed, the organisms being present in greater proportion than in the intestinal fluids of severe cases of infection.

RECAPITULATION.

Boric acid, eucalyptol, ichthyol, oil of cassia, and infusion of quassia had slight if any effect on the amœbæ.

Tannic acid 1–100, sulphate of copper 1–2,000, permanganate of potassium 1–4,000, and sulphate of quinine 1–1,000 had a distinct, moderate effect on the growth of the amœbæ and spirilla within thirty minutes.

Benzoyl acetyl peroxide, acid succinic peroxide 1–1,000, permanganate of potassium 1–2,000, sulphate of quinine 1–500, nitrate of silver 1–2,000, argyrol 1–500, and protargol 1–500 exercised a very marked effect on the growth of the cultures within thirty minutes, and in the case of the silver salts and the acid succinic peroxide the action was plainly due to the destruction or inhibition of the growth of the symbiotic cholera spirillum.

Thymol 1–2,500 applied for fifteen minutes had the unique effect in some of the experiments of destroying the amœbæ while exercising only a moderate effect on the cholera spirilla.

There is no specific treatment for amœbic dysentery, but, if the test-tube results detailed above are a fair index of the behavior of the substances in the actual local treatment of the disease, the clinician can add to his therapeutic armamentarium a few more agents of a value equal or superior to quinine. Such a choice will be appreciated by physicians practicing in the Tropics when they encounter patients intolerant of quinine, or otherwise failing to benefit by its local action.

THE PATHOLOGY OF INTESTINAL AMOEBIASIS.

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In former papers, one of us (Musgrave), *Bulletin No. 18,* has dealt with the subject of the cultivation and etiological significance of amœbæ. The following remarks will be limited to the pathology of the intestinal amœbic disease:

It may be well to state at the outset that we can see no valid reason for departing from the nomenclature of Lösch. He described a pathogenic amœba and called it *Amœba coli.* Why this term should be applied to a supposititious nonpathogenic organism it is difficult to say. We shall in referring to the cause of intestinal amœbiasis use the name introduced by Lösch.

Among the many articles in the literature of amœbiasis there are but few which are of special value from the pathologic side. Chief among these are those of Councilman and Lafleur (*Johns Hopkins Hospital Reports, 1891,* II, 395), Harris (*Amer. Jour. Med. Sc., 1898,* CXV, 384), Howard (*Buck's Reference Handbook, 1900*), and Rogers (*Brit. Med. Jour., 1903,* I, 1315). Taken together, these works give a very complete picture of the disease as we have seen it.

Material.—This has been obtained from various sources, among which have been the First Reserve Hospital, Bilibid Prison, and the Civil Hospital, all in Manila. Other material has been obtained from the service of Dr. Strong and the private practices of Drs. McDill and Musgrave, also of Manila.

Methods.—Bits of tissue from autopsies were fixed in Zenker's solution, in absolute alcohol, Flemming's solution, and Kaiserling, and ultimately imbedded in paraffin.

Sections from alcohol tissues were stained by Mallory's thionin and oxalic method, those from Flemming's solution in safranin and

safranin-picro-indigo-carmin, those from Zenker's in magenta-picro-indigo-carmin (Borrel), gentian-violet-picro-indigo-carmin, eosin-methylene-blue, eosin and toluidin blue, hematoxylin and eosin, hematoxylin and picro-fuchsin (van Gieson), chloride of iron hematoxylin (Mallory), and Heidenhain's iron hematoxylin.

After some preliminary staining it was evident that for simple diagnosis from alcohol tissues, the eosin-toluidin-blue and thionin-oxalic-acid methods were most satisfactory in the order given; for other (sublimate or chrome) tissues, hematoxylin and eosin were most useful. However, it was evident that for careful examination and cytologic study the best results could be obtained with Heidenhain's iron hematoxylin and Borrel's stain, or in the case of Flemming's solution sections with safranin-picro-indigo-carmin. Borrel's method may be modified by using gentian violet in the place of magenta with excellent results.

Borrel's staining gives most brilliant results in sublimate tissues, producing very clear, distinct pictures, which are only surpassed in clearness and delicacy by Heidenhain's iron hematoxylin. Both of these stains have the additional advantage of revealing the bacteria, when differentiation is properly carried out.

Hematoxylin and eosin is a very satisfactory routine method for demonstrating the amœbæ, although the contrasts are not so distinct and the finer elements can not be so well demonstrated.

Harris's method, when applied to sections of the intestine, gives a considerable contrast between the amœbæ and other cells. By its use the organisms are easily found with the lower powers of the microscope.

Kaiserling tissues respond best to hematoxylin and eosin, but are of little comparative value for detail, and have been used almost exclusively to demonstrate the lesions in a gross microscopic way. From such tissues serial sections have been made of various types of lesions and their form and extent studied in that way.

Gross lesions.—Many writers say that the macroscopic lesions of intestinal amœbiasis are pathognomonic. While in certain cases—perhaps in the majority—this is true, there are others in which the picture may be very deceptive. We have seen cases from whose appearance at autopsy we could not say definitely whether or not we were dealing with amœbiasis, tuberculosis, or some other ulcerative condition, and others, few to be sure, in which the majority of the ulcers were not of the classical undermined type, the

undermining being either incipient or obscured by the process of healing. However, and this is not uncommon, a truly pathognomonic picture is presented when the mucous membrane shows all the types of lesions, and in which the walls of the gut are thickened and œdematous.

The various lesions many not be sharply distinguished, for the process is a progressive one, and one type shades into another very gradually, so that only for purposes of convenience in description we shall arbitrarily designate the various stages of the gross process.

I. *Preulceration.*—This stage is characterized by the presence of the "small raised dots" of Rogers, which vary in size from 0.5 to 2 millimeters in diameter and are intensely congested. As a matter of fact, when studied macroscopically, they are seen to be composed of one or more capillary hemorrhages into the intraglandular tissue. Usually associated with this condition is one of erosion of the superficial layers of the mucous membrane. However, these erosions may be encountered in the absence of any marked congestion, although a moderate injection, at least, is the rule. With both of these processes there is little of the marked thickening of the submucosa which is so constantly seen in the more active ulcerative stages of the disease. These early lesions may be seen in any portion of the affected gut. By using the sigmoidoscope we have demonstrated them low down in the rectum within a couple of inches of the anus. They are most frequently encountered and are most numerous in the more acute cases, but may also be seen in chronic ones.

II. *Ulceration; (a) type of Harris.*—These lesions, though rarer than the classic type, are nevertheless not uncommon. They are possibly the result of the process of erosion mentioned above and are primarily confined entirely to the mucous membrane. As Harris says, they "generally reach into the submucosa and rarely to the circular muscle, but never deeper." They probably commence as a very circumscribed erosion and spread laterally as rapidly as they do downwards. Macroscopically the edges are abrupt, sometimes giving the ulcer a "punched-out" appearance. They are round or oval in form and their edges are usually thickened and marked by intense congestion. Their bases are comparatively clean, grayish, and œdematous. They are often situated on the apices of intestinal folds and have a tendency to increase in the direction of the short axis of the bowel. This type of ulcer has a general distribution and may be encountered in any part of the bowel. It is

less frequently seen in the more advanced and very chronic stages. The fact that it is most common in those bowels showing the preulcerative lesions speaks for its being intermediate between the very early petechial lesions and the undermined ulcer. This is the type most common in the ileum.

(*b*) *Classic or undermined ulcers.*—These are seen in an early stage, as minute yellowish or grayish spots in the mucosa of the bowel, frequently at the centers of the petechiæ spoken of as the preulcerative stage of the disease, and are usually surrounded by a zone of congestion more or less well marked, as the case may be. These spots represent the mouths, filled with necrotic material, of passages leading to larger or smaller cavities in the submucosa, which are also filled with the same material. As the process extends, the pocket in the submucosa is enlarged parallel with the surface in all directions, and although the necrobiosis eventually involves all the coats of the bowel, the muscular layers and the mucous membranes suffer less rapidly, so that there results an ulcer with its base on the circular muscle and with overhanging edges of mucous membrane. Such ulcers may be of sizes varying from that of a pinhead up to that of the palm of the hand, and may occur in any part of the large intestine and even in the lower part of the ileum, though in the latter they are smaller.

During the process of ulceration, the submucosa becomes generally thickened and œdematous, as may also the muscular layers and the peritoneal coats.

During extension, such ulcers may coalesce beneath or upon the surface, and it is no rare thing to find even small submucous pouches communicating with each other by tunnels, while the mucous membrane may show no more than a catarrhal condition.

In many of the larger lessions, the circular muscle fibers are exposed, forming the base of an ulcer, and shreds of this may be seen nearly separated from the rest and may be removed by gently scraping. In still more extensive ulceration the muscular layer may become necrosed or even perforated and the ulcer may then be bounded externally by the peritoneum or omentum.

There are, perhaps, few diseases in which the omentum plays so imported a protective part as in the one under discussion. Very early in the ulcerative stages this membrane may be found plastered upon the peritoneal surface of the gut in preparation for the accidents which may follow.

Because of this function of the omentum, localized suppurations are common. Ulcers may also perforate into the subperitoneum at almost any point, the abscess as a usual thing remaining circumscribed, although it may burrow widely. One case of this class of burrowing abscesses had perforated in the retroperitoneum in the cæcal region and had extended upward and perforated into the right pleural cavity.

III.—*Healing.*—As healing takes place, the mucous membrane gradually extends from the margins, so that in the early stages the ulcer seems to be lined with epithelium, except upon its base. In the case of very small ulcers there may be complete repair, in other more advanced cases there is considerable formation of scar tissue which may lead to contractions. This we have not seen. The commonest outcome in cases of long duration, especially in those not treated systematically and continuously, is the establishment of a chronic catarrhal condition with subsequent atrophy—a condition of *enteritis chronica atrophicans.* This is recognized in various parts of the world as sprue or psilosis. It is not a result of amæbiasis only, although it may be so in imperfectly treated cases. The gross features of the bowel in such a condition are thinness, absence of normal folds, atrophy of the mucous membrane, and increased length. Combined with this atrophic condition may be one of localized hypertrophy, resulting in the formation of more or less well-developed polypi.

Epitome of the gross appearances.—In a general way the process studied is as follows: In the early stages of the disease there occurs a catarrhal condition of the mucous membrane with hypertrophy and cystic and mucoid degeneration. At various points, from the lower part of the ileum to the lower part of the rectum, small raised hemorrhagic spots occur, which later lose their mucous coverings and resemble erosions, and later still ulcerate. If abscesses have formed in the submucosa, they rupture into the bowel and produce the early step of undermined ulcers. The ulcers resulting from simple erosions are not, as a rule at least, of the undermined type, but they may become so. Undermined ulcers are usually, while the cause persists, progressive.

When secondary infections occur the processes of the disease may be modified by diffuse congestion, hemorrhagia, diphtheritis, or gangrene. Perforations may occur in the course of the ulcerative

stages of the disease with resulting localized or general peritonitis, retroperitoneal abscess, etc.

The healing of small lesions may take place with complete repair, or of large lesions with the formation of scar tissue and with subsequent contractions. Peritonitis may result in the formation of adhesions. Complete cure may be the eventual outcome, or a condition of chronic atrophic enteritis or chronic catarrhal enteritis may result.

Generally, and probably always in active cases, the intestine is thickened. This increase may be due to edema of all the layers, but chiefly to that of the submucosa. It may also be caused by the presence of the abscesses and sinuses which are so common in that layer. In very active cases the subperitoneal coat may be very much thickened, mostly from œdema. The mucous membrane between the ulcers in uncomplicated cases usually appears normal.

Distribution of the lesions.—There seems to be only a partial unanimity regarding the extent and localization of the lesions in the disease. To quote recent remarks on the subject, Futcher (*Journal of the American Medical Association, 1904*) says the rectum in a majority of cases is not involved or is so, only to a slight extent. Harris (loc. cit.) remarks that in fully half the cases the lesions do not extend above the beginning of the transverse colon, and Rodgers (loc. cit.) writes that almost invariably the lesions are more marked in the cæcum and ascending colon and frequently limited to these areas.

In considering the presence of lesions above the ileocæcal valve, Futcher says that in two of 119 cases slight superficial ulceration was present in the last few centimeters of the small intestine. Rodgers concludes that "the process never invades the ileum, which is contrary to frequent results in other forms of dysentery." Many writers speak of the more or less frequent involvement of the appendix. Such observations taken from literature might be multiplied many times, but these serve to show how different are the results of careful and accurate observations based upon studies carried out in different countries and even in different parts of the same country.

Our statistics are based upon two series of cases, each of which comprehends 100.

Series A: Composed of cases that received either unsystematic treatment by enemas or none at all. This series is one in which the cases were received in the pathological service of Dr. R. P. Strong at the First Reserve Hospital and Army Pathological Laboratory, and in which the necropsies were performed by Drs. Strong and Musgrave. Dr. Strong kindly turned over to us the autopsy records for examination and study.

Series B: Composed of cases treated by rectal injections.

SERIES A.

Entire large bowel involved (except extreme lower part of rectum)	87
Lesions confined to cæcum and ascending colon	5
Lesions confined to transverse colon	1
Lesions confined to descending colon, sigmoid, and rectum	0
Not recorded	7
Total	100
Appendix ulcerated (with large intestine)	6
Ileum ulcerated (with large intestine)	2

SERIES B.

Entire large bowel involved	72
Cæcum and ascending colon	18
Descending colon, sigmoid, and rectum	9
Tranverse colon	1
Total	100
Appendix involved (with large intestine)	8
Ileum involved (with large intestine)	5

The variations shown in the two series, and those in literature as well, perhaps, may be at least partly explained. The factors to be considered are the duration of the disease at the time of death, the kind of treatment, its duration, and the stage of the disease in which the patient was when treatment was commenced.

If we take the Johns Hopkins Hospital series as an example, we may be reasonably sure that the majority, if not all, of them were treated by irrigations per rectum, as in our cases of Series B. A rational consequence of this might be that in many cases the lower lesions in the bowel had healed, and at autopsy only those that were inaccessible to treatment or which irrigations had not reached would be seen. This undoubtedly accounts, in part, for the variations manifested in our series and this dissimilarity would undoubtedly be more prominent had a larger number been properly, consistently, and persistently treated.

The duration of the disease at the time of death is unquestionably an exceedingly important factor in untreated cases and in those in which irrigation has been neglected. It may be said that without treatment the greater the duration of the disease the more extensive is the distribution of the lesions.

For the reason indicated above and for other obvious ones it may be said that the findings at necropsy can not be taken as a guide to

the distribution of the lesions at any period save that immediately preceding death.

We have selected from our series twenty-five cases in which death occurred from intercurrent disease early in the amœbic process and find that among these there is an increased proportion of cases showing ulceration confined to a portion of the intestine and a coincident decrease in the number showing ulceration throughout the large gut:

Ulceration confined to the cæcum and ascending colon	11
Ulceration confined to the descending colon, sigmoid, and rectum	8
Ulceration throughout the bowel	6
Total	25

Lesions of the small intestine, in our experience, have always been confined to the lower ileum and have been the apparent result of direct extension from the cæcum, which is usually severely ulcerated in these cases. We have had one in which ulceration extended 32 centimeters above the ileocæcal valve, but ordinarily the lesions consist of one or a few ulcers immediately above, or within 5 centimeters of, the Bauhinian valve. However, when there is a diphtheritis of the cæcum, the membranous exudate more often extends above the valve for a longer or shorter distance, but without ulceration. In such cases amœbæ have not been demonstrated and consequently they have not been included in our statistics.

As with the small intestine, so with the appendix, only cases with distinct ulcerative processes, in which amœbæ were demonstrated, have been included in our statistics. In many necropsies this organ was found clinically diseased from causes other than amœbiasis and it occasionally showed an acute process which could not be proved to be due to amœbæ. All such cases have been excluded from the tables.

Histology.—In general, it may be said that the mucous membrane between the ulcers is but little changed. In many places no deviation from normal can be noted. However, in the immediate neighborhood of the lesions there is a tendency to hypertrophy with mucoid degeneration and even cyst formation. The latter is less common in the cases we have studied than in those reported by Councilman and Lafleur. The most common change in the mucosa is in the immediate vicinity of the lesions and is shown by a tendency to more diffuse staining than is seen in normal epithelial

cells; and whenever this, perhaps a sign of incipient coagulative necrosis, is visible, there is usually some distortion of the glands beneath the surface. Such changes are most frequently seen in the very early stages. In many cases the cells lining the glands are separated from the basement membrane and lie singly or in clumps in the lumen. Under such circumstances it is not uncommon to see amœbæ in the glands, lying among the desquamated cells or forcing their way between the epithelia and the basement membrane.

The most marked feature of the early lesions is congestion, often combined with capillary hemorrhages which are most noticeable immediately beneath the mucosa. This congestion may extend even to the submucosa, in which layer there is also a certain degree of thickening, chiefly due to œdema. Together with congestion, there is an increase of cellular elements of the lymphoid type in the interglandular tissue. The muscularis mucosa at this stage of the disease may show no changes or only a slight œdema.

The most interesting feature of this early process lies in the distribution of the amœbæ. Not only may they be seen in the glands, as described above, but they may also be present, sometimes in large numbers, in the interglandular tissues and blood vessels, in the muscularis mucosa, and in the dilated veins of the submucosa, and this, with changes scarcely perceptible, if only the low powers of the microscope are used. In such lesions bacteria are very few in number and often can not be found even after prolonged search, and none can be demonstrated in the amœbæ. The latter, even in the blood vessels, show the peculiar rod-shaped or crystalline bodies which stain intensely with magenta and hematoxylin, the radiate structure of the ectoplasm and ingested cells.

In sections from lesions slightly more advanced and showing a more extensive, though still superficial necrosis, the glands immediately surrounding them are hypertrophied and the cells show mucoid degeneration. There is the same lymphoid infiltration, with, if anything, a greater congestion. In the congested area each gland mouth seems to be surrounded by a zone of hemorrhages. The cells of the necrotic mucous membrane are incorporated, with occasional leucocytes, granular detritus, amœbæ, and bacteria, into a more or less well-formed membrane. There is a more extensive separation of the glandular cells, and in such glands amœbæ can usually be seen either in the lumens or between the cells and the basement membrane. Within the interglandular connective tissue

amœbæ may also be seen in the blood vessels and lymph spaces. As the process progresses and as the lesions become more advanced the effect upon the submucosa is more marked. The congestion is augmented, the edema is increased and the number of amoebae is greater. There is usually also an increase in the mucoid changes of the epithelium surrounding the lesion. In a few cases we have seen a very low, atrophic mucous membrane and a comparatively thin-walled gut, although there were extensive ulcerations. It is reasonable to suppose that we were dealing in such cases with an infection of a bowel previously the seat of a chronic enteritis. In all the cases we have studied, regardless of the state of the mucous membrane or submucosa, we have seen an extensive lymphoid cell infiltration and at least a moderate hypertrophy of the lymphoid apparatus.

In all lesions, whether early or late, the character of the cellular infiltration is the same in uncomplicated cases. It seems, if amœbæ can be demonstrated in the tissues and if at the same time there is a polymorphonuclear infiltration, that bacteria are playing an active part in the process, especially if with the infiltration there is any degree of nuclear fragmentation. In certain cases infiltration with polymorphous leucocytes may be seen about the margins of the ulcers, although at the base of the lesions and in the submucosa they were present in but inconsiderable numbers. In some cases, although there were some or even many, bacteria present, there was no process which seemed to be directly attributable to these. It is possible that in such cases these were simply the nonpathogenic, harmless commensals of the amœbæ. In others, bacteria seemed to play at least as important a rôle as the amœbæ, noticeably in those in which there was diphtheritis and gangrene. It may be that the bacteria play an important part in determining whether or not hemorrhages shall occur, for it is certain that in uncomplicated cases thrombosis is a common and early occurrence. In many very early stages the interglandular vessels of the submucosa may be seen generally thrombosed. If an ulcer is filled with a diphtheritic slough which is carried away suddenly, the chances of the hemorrhages are much increased, as is the case in typhoid.

Usually the necrotic process extends for some distance beyond the ulceration and often beyond the amœbæ, but in many instances amœbae seem to be present in healthy tissue, notably in the early

lesions. It is a question whether this necrobiosis is the result of some secretion of the amœbæ or whether it depends more upon thrombus formation. In some places the latter seems to be the predominating factor, in others the former, while in still others neither appears to influence the process. It is, however, certain that the thrombosis assists the amœbæ in extending their zone of action, as it may likewise assist the bacteria.

Necessarily the contents of the ulcers vary according to the degree of ulceration and to the character of the bacteria present. In uncomplicated cases, which microscopically show a rather clear, yellowish, gelatinous material in the opening, the ulcer contents are composed of a granular base of albuminous character, in which cells in various stages of degeneration are imbedded, together with amœbæ, bacteria, and usually a few red blood corpuscles.

In all the lesions the amœbæ vary widely in size. Measured with a Zeiss schrauben micrometer they range between 4 and 35 μ.

In the earliest stages of the amœbic invasion, the œdema effects the submucosa. In the later ones the subperitoneal coat is also involved and adds considerably to the thickness of the gut. Eosinophiles are not uncommon in either the modified or unmodified amœbic process. They occur for the most part in tissues at some distance from the lesions, usually in the neighborhood of blood vessels, and are not uncommonly encountered in the subperitoneal connective tissue, when that layer has become œdematous. These are perhaps more numerous in the secondary infections, as are also mast cells. Plasma cells are frequently seen in the submucosa. In the most extensive ulcerations the picture is modified only by the extent of the process. Whether the ulcers are undermined or not, there is always the same appearance of coagulative necrosis, with lymphoid infiltration, congestion, and thrombosis, and comparatively little polymorpho-leucocytic invasion.

The two most evident features of the intestinal lesions, when viewed with a comparatively low power of the microscope, are the necrobiosis and the relative infrequence of leucocytes, features which suggest the important rôle of the amœbæ, for ordinarily in bacterial infection there is an associated local leucocytosis of varying intensity. A point of some importance is brought out by the fact that in the very early lesions, the preulcerative ones, the amœbæ may be encountered not merely in the glands but beneath the epithelium

and within the lymph spaces and blood vessels of the interglandular tissue and the submucosa.

The character of the amœbæ in sections.—In toluidine blue and eosin the estosarc stains fairly definitely and the vacuoles of the protoplasm show well. The bacteria also stain, as do the encysted bodies and fragments. The nucleus is very definitely stained and surrounded by a more or less distinct perinuclear space. The nuclear membrane colors a clear deep blue. The nuclear protoplasm appears pink and contains one or several deep blue-black bodies, or perhaps none. There may be several dark-stained thickenings in the nuclear membrane. As a rule, in well-stained sections the amœbæ, where they do not contain much extraneous material such as bacteria and nuclear detritus, are less deeply stained than the cells of the intestinal mucous membrane. The protoplasm of those which are deep in the tissues is less intensely colored than that of the ones in the mucous membrane, and it may be that this phenomenon is due to the fact that in the more superficial layers the organisms have taken up more mucous material. That there is some reason for this supposition is shown by the fact that this stain is much less useful in studying the organisms in liver abscesses, where of course there is no mucus.

Perhaps the most brilliant stain for amœbæ in tissues is that of Borrel. This consists of—

I. Saturated aqueous solution magenta red.
II. Saturated aqueous solution picric acid.
Saturated aqueous solution indigo carmin aa.

Stain with No. I for twenty minutes and wash.

Stain with No. II for five minutes, wash, and differentiate with alcohol, xylol, and balsam.

With this, the amœbae are not so readily distinguished by the low powers of the microscope as with the thionin or eosin toluidine-blue stain, but the finer organization is much more easily studied with high powers. Generally with this stain the amœbæ are less deeply colored than the surrounding tissues, being a rather pale bluish or purple, or, in well-decolorized specimens, of a greenish hue. The edge of the organisms shows as a fine blue line, which is more distinct about the body of the parasite and less so about the pseudopodia. The ectoplasm appears as a finely reticular or almost hyaline substance, the fibrillar or granular part of which is stained a very faint blue. The endosarc appears as a granular material

more deeply stained than the ectosarc, and purplish, bluish, or greenish, according to the degree of decolorization. Within this are spaces which remain uncolored, though in thick sections they have a bluish tint due to the underlying stained material. Bacteria may also be in the endosarc. The rods of which Councilman and Lafleur speak, which are not seen in all cases, occurring in those in which there is more extensive sloughing or diphtheritis, may also be seen. It may be that these are crystalline, derived from the blood, or Charcot-Leyden crystals, which are occasionally found in leucocytes in certain conditions and which Askanazy says are oxyphilic (*Munch. Med. Woch., 1904,* LI, 1945).

The nucleus may be surrounded entirely or in part by a clear perinuclear space. The general color of the nucleus is violet or purplish. The outline is sharp, and, if the section is not too decolorized, should assume a clear crimson tint, in the form of a more or less incomplete ring, or it may be nodulated on its inner surface corresponding to thickenings in the chromatin. There are also occasionally crimson granules within the nucleus and sometimes one perfectly round mass corresponding to the nucleolus.

Ingested cells, such as red blood corpuscles, leucocytes, etc., may also be seen within the protoplasm in various stages of degeneration, the stain depending upon the extent of the process.

In sections so decolorized that the magenta is all removed, the nucleus appears of a blue color, deeper than the tint of the rest of the cell and with the chromatin material still more deeply stained.

Heidenhain's iron hematoxylin is as excellent a stain for amœbas as it is for other tissues, although not so brilliant as the magenta-picro-indigo-carmin. In carefully manipulated sections the nucleus of the amœbæ is somewhat more deeply stained than the cystoplasm and ordinarily appears as a dense blue-black ring, in the center of which is the round black nucleolus. The nuclear plasma is usually merely of a blue tint and is divided by a mesh of delicate dark reticulum. There may be other deeply stained chromatic elements or granules within this. The radial striæ of the cytoplasm is well shown and the crystalline bodies are, when present, an intense black. The spongioplasm appears as a network of dark lines and the cell boundary is sharply differentiated. Red cells, when present, stain according to the state of degeneration, those most recently ingested being black; those least so, yellowish. Bacteria, when present, stain sharply and distinctly when not too far degenerated or digested.

Amœbæ grown in cultures from amœbic ulcers, when stained by Borrel's method, show somewhat different reactions.

Cover-glass impressions may be made in the following way:

A cover glass is placed upon the growths on agar plates, removed quickly, and instantly plunged into a very hot saturated solution of mercuric bichloride. It is then washed in Gram's solution or a weak tincture of iodine and rinsed in 80 per cent alcohol, after which it is washed in water. The stain is then applied in the usual way. After this process the preparations are differentiated in alcohol, cleared in xylol, and mounted in xylol-damar. The amœbæ are seen in various conditions, as they were in the culture, and of various shapes and sizes. In many the pseudopodia have not been withdrawn and can be well seen. Under these circumstances the ectosarc has a pale, diffuse blue color, or is perhaps very finely granular and has a very sharply differentiated limiting line. However, usually this is not well seen and the whole organism has a sharply circumscribed, blue, granular appearance. The contractile vacuole shows clearly as an unstained ovoid or oval space, usually near the surface of the organism but occasionally near the nucleus.

The nucleus is composed of a round, central, deep purple body, surrounded by a narrow, pale-bluish, homogeneous zone, and this in turn is surrounded by a denser, blue, granular one. About this in turn is ordinarily a second more or less faintly stained zone. The whole nuclear body is round or slightly oval. The nucleolus is always round.

Comparing the amœbæ in such preparations with those in sections, we can readily see that the relative size of the nucleus is the same, as is also the relation between the protoplasm and nucleus; but in the tissues the contractile vacuole is usually not so distinct and the nucleus does not present the same appearance. This may be due to the fixation or it may be due to the different degree of decolorization. The dissimilar nutritive conditions may also affect the microchemical staining reactions.

The small amœbæ, having the size of an erythrocyte or smaller, should show the same relation in the size of nucleus and cell body.

The distinguishing features of the amœbæ are their generally irregular or oval shape (though they are often round), their relatively small, round nucleus, and the larger amount of granular or vacuolated protoplasm, which often contains foreign bodies.

Relation of the amœbæ to the tissues.—The mucous membrane: It is not known whether the amœbæ are able to attack or pass through an intact mucous membrane; in fact, it seems probable that in order for them to enter the deeper layers of the intestine there is some change from the normal (Schaudin). It is possible that catarrhal conditions, however slight, are accompanied by erosion, or necrosis of even a few of the superficial cells, and would offer the necessary conditions for invasion. It is at any rate certain that the amœbæ have less influence on the epithelial cells than on the supporting tissue in many glands. That this is true may be demonstrated repeatedly. The amœbæ may be seen either in the lumen or between the lining cells and the basement membrane. As a rule in these cases the epithelium detached has lost no more of its normal character than might be expected, and if adherent it seems more healthy than would be imagined in the presence of an organism whose power of causing cellular destruction is as great as it is in the case of the amœba. Generally speaking, epithelium seems to have as great resistence to the amœba as has muscular tissue.

Muscle: In all works on amœbiasis attention has been called to the lack of resistance the connective tissue offers to the progress of the organisms. Attention has been repeatedly called to the fact that the extensive ulcerations are in form and situation dependent upon this quality in the submucosal layer of the intestinal wall. The priliminary feature of the changes in this position is œdema, which, after invasion by the amœbæ, is followed by swelling of the fibers and cells, infiltration with round cells, and lymphocytes, sometimes accompanied by fibrin formation. The nuclei of the swollen cells become paler, the appearance of fibrillation is lost, and the tissue becomes hyaline or necrobiotic. A further feature of the process is that new tissue is found early and this has the appearance of granulation tissue. Suppuration, as previously indicated, probably does not occur in the uncomplicated disease.

Blood vessels: Just how the organisms enter the blood vessels can not be satisfactorily stated. In some sections they are seen in large numbers, in the same or others they may be in the perivascular lymph spaces, or they may appear immediately beside the endothelium of the capillaries. It is possible that they enter directly through the capillary walls by virtue of their inherent

power of progression, in much the same way as the leucocytes wander in and out. There is the possibility that in the process of tissue destruction the vessel walls are so changed as to make this process more practicable, or that, following capillary hemorrhages and before coagulation has occurred, the organisms may enter the vessels and make their way along. Certain it is that they do not necessarily cause thrombosis by their presence, although this appearance is by no means uncommon in tissues, and especially in the vessels of the interglandular tissues. So far as the blood cells are concerned, the amœbæ are able to ingest and destroy apparently healthy erythrocytes and leucocytes.

Relation of amœbæ to the cells.—It was shown by Councilman and Lafleur, and forcibly insisted upon by Howard, that leucocytic infiltration is not a feature of amœbiasis. On the contrary, it is stated that the process is more in the nature of a subacute or chronic inflammation, in that the cells predominating in the infiltration are formative ones and lymphocytes. In addition to these last-mentioned cells there is often a considerable number of eosinophiles, though this is not the rule. However, if the condition is a chronic inflammatory one, then this is the type of infiltration we should expect, for lymphocytes and eosinophiles are the cells which, par excellence, occur in such pathologic states (*Muir. Brit. Med. Jour., 1904,* ii, 585).

In one series of sections we saw considerable numbers of mast cells, which occurred chiefly in the glandular layer of the bowel. Upon what conditions the presence of these depend we can not say, except to note that in the bowel from which the sections were made there was considerable diphtheritis.

Plasma cells are not uncommonly seen in the submucosa, as Councilman and Lafleur state. If these are of lymphoid origin, we should expect them to be frequently met with in subacute or chronic inflammation in which there is a proliferation of, and invasion with, lymphoid cells.

Relation of amœbæ to bacteria.—From our experience it can not be said that the presence of bacteria limit the field of activity of the amœbæ. As a matter of fact, the organisms seem to be about as numerous in cases complicated by bacterial superinfections as in uncomplicated ones, unless it be in those in which pyogenic cocci are present. In one case so complicated there were certainly fewer

amœbas in the lesions and there was very active leucocytic infiltration with marked coincident karyorhexis, a very uncommon picture for amœbiasis.

When amœbæ are found in exudates rich in bacteria they show evidence within their bodies of a very active phagocytosis.

CONCLUSIONS.

I. Intestinal amœbiasis is a peculiar ulcerative condition of the intestine caused by *Amœba coli* (Lösch), usually confined to the large intestine, though occasionally (7 in 200 cases) the ileum is affected and more often (14 in 200 cases) the appendix is involved.

II. In the majority of cases the condition affects the entire bowel (159 in 200 cases), though it may be limited to one or more portions, most commonly the cæcum and ascending colon (23 in 200 cases).

III. The ulcers show a tendency to be undermined, due to the lack of resistance on the part of the submucous layer of the bowel.

IV. The organisms may enter the blood vessels very early in the disease and may be transported to the submucosa without lesions of the muscularis mucosa.

V. The disease is a subacute chronic inflammatory process, as shown by the character of the exudate and infiltration, by the early formation of granulation tissue, and by the absence of leucocytic infiltration.

VI. Complete healing may be accomplished, or a condition of chronic atrophic enteritis or chronic catarrh may persist, which is known as sprue or psilosis.

ILLUSTRATIONS.[1]

PLATE I. Colon. Thin-walled gut, with shallow ulcers, some slightly undermined, others punched out.

II. Sigmoid. Irregular ulceration with diphtheritis.

III. Colon. A moderately thickened gut with various types of ulcers.

IV. Cæcum. Marked degree of disorganization of the bowel with shreds of muscularis and submucosa. Perforation.

V. Rectum. Extensive ulceration and diphtheritis. Thick-walled gut.

VI. Colon. Various stages of ulceration.

VII. Colon. Extensive distribution of punched-out ulcers, some slightly undermined.

VIII. Colon. Thickened bowel with some large ulcers and some very early ones.

IX. Early intestinal lesion. Shows superficial necrosis, glandular distortion, and round-cell infiltration. Borrel's stain. (Zeiss objective AA, compensation ocular No. 4, bellows at 30 centimeters.)

X. Early lesion. Extending necrosis, destruction of glandular epithelium, invasion of amœbæ, and round-cell infiltration. Borrel's stain. (Zeiss objective AA, compensation ocular No. 4, bellows at 50 centimeters.)

XI. Submucosa in an early lesion. Borrel's stain. (Zeiss objective AA, compensation ocular No. 4, bellows at 30 centimeters.)

XII. Thrombosis of blood vessels of the mucous membrane of the colon. Borrel's stain. (Zeiss objective DD, compensation ocular No. 4, bellows at 50 centimeters.)

XIII. Amœbæ in the muscularis mucosa. The section is the same as that shown in Plate X. (X 500.)

XIV. Amœbæ in an area of hemorrhage in the submucosa. Borrel's stain. (X 500.)

XV. Amœbæ in a blood vessel. (X 500.) Heidenhain's iron hematoxylin.

XVI. Amœbæ in a blood vessel. Borrel's stain. (X 500.)

XVII. Amœbæ in the lymph spaces of the submucosa. Borrel's stain. (X 500.)

XVIII–XXI. Amœbæ in tissues. Borrel's stain. (X 1400.)

XXII. Amœbæ from a culture. Impression preparation. Borrel's stain. (X 500.)

XXIII. Ibid. (X 1400.)

[1] The photographs and photomicrographs were made by Mr. Martin, the photographer of the Bureau.

PLATE I.

PLATE II.

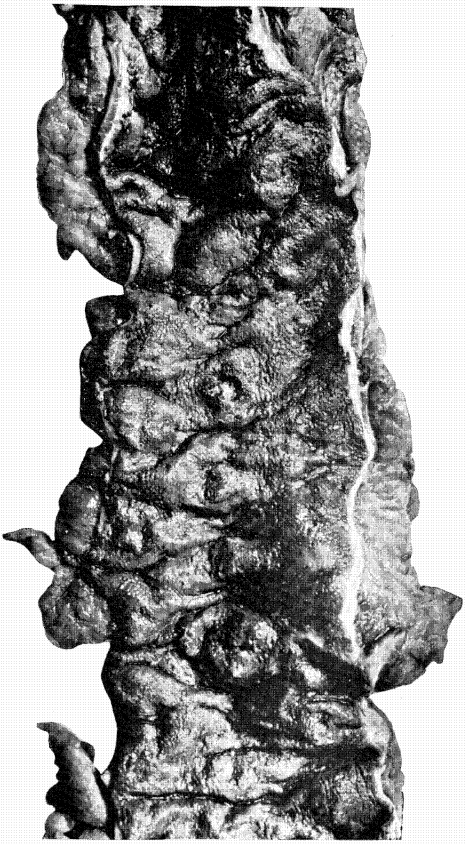
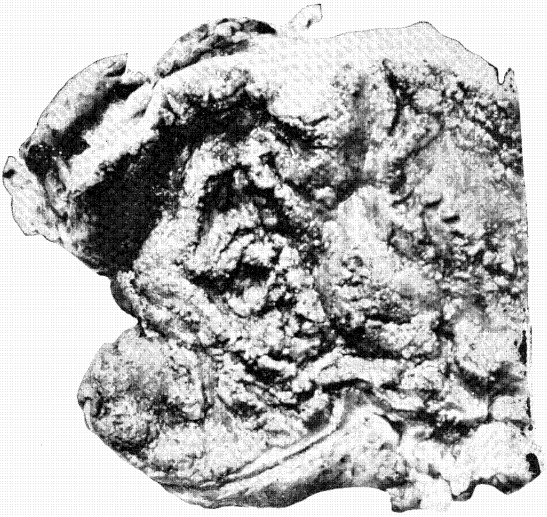


PLATE III.

PLATE IV.



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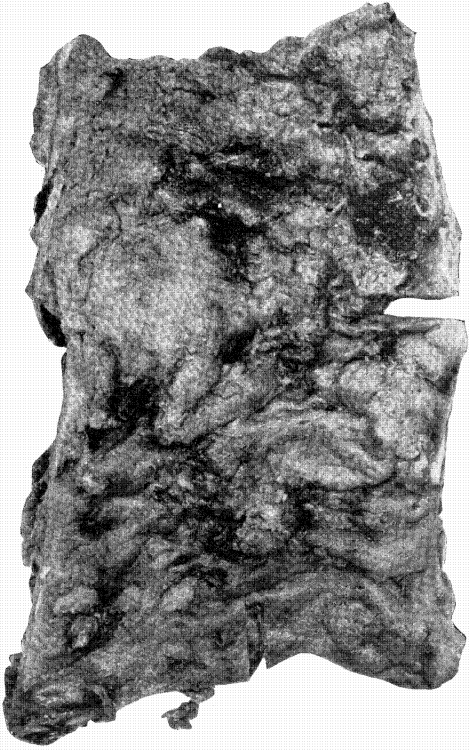
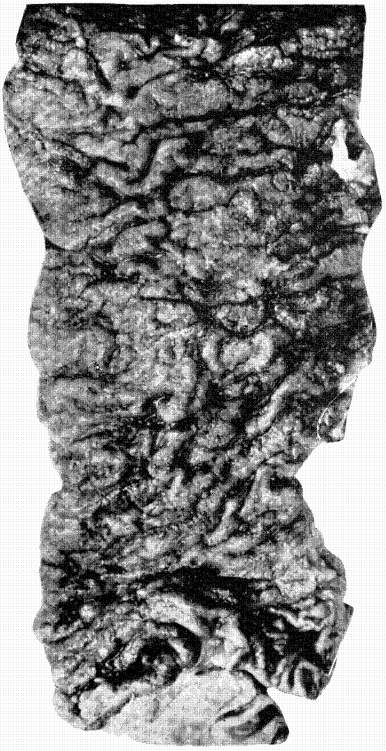


PLATE VI.

PLATE VII.



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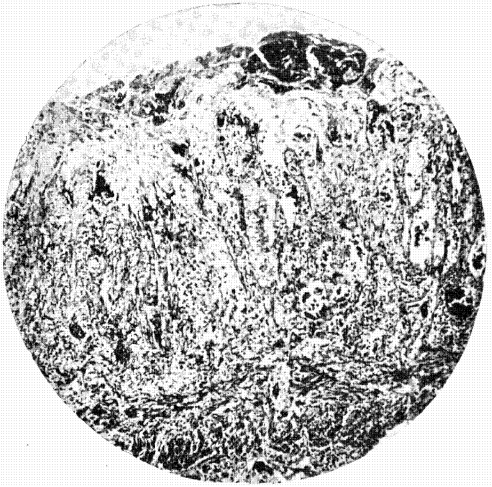

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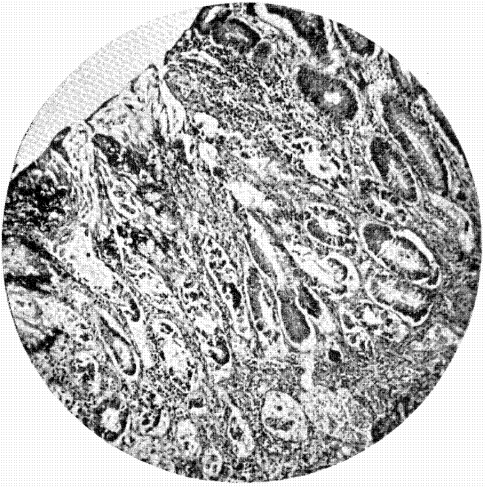

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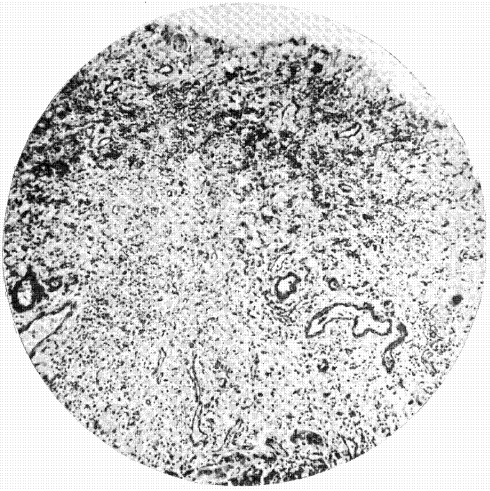

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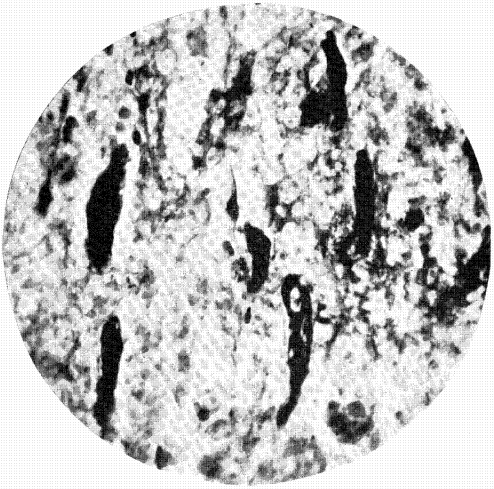

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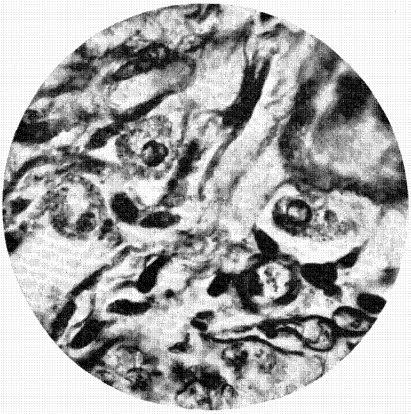

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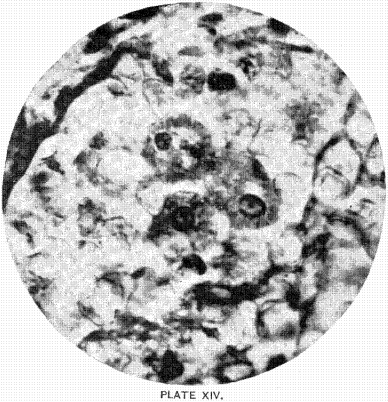

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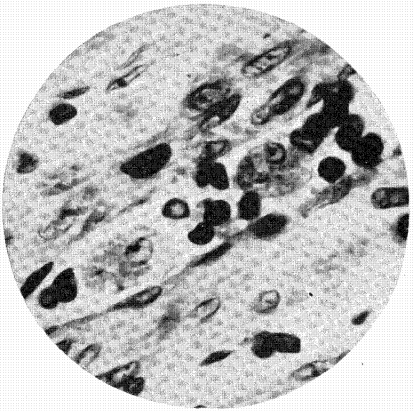

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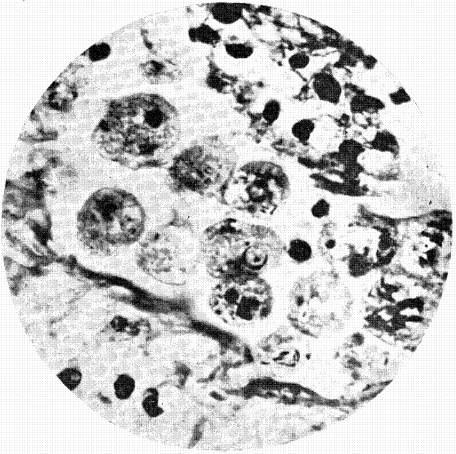

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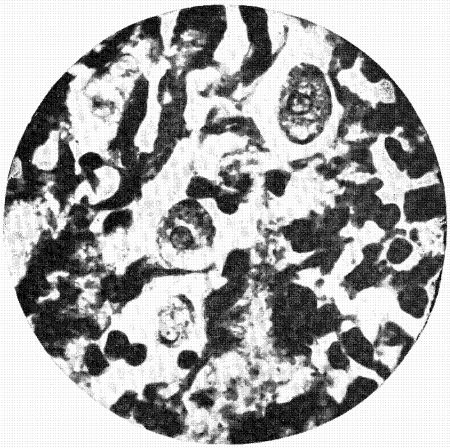

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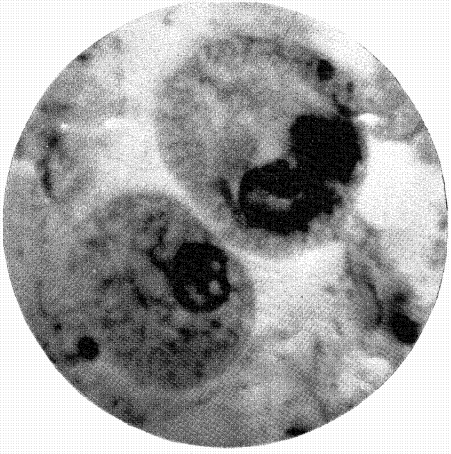

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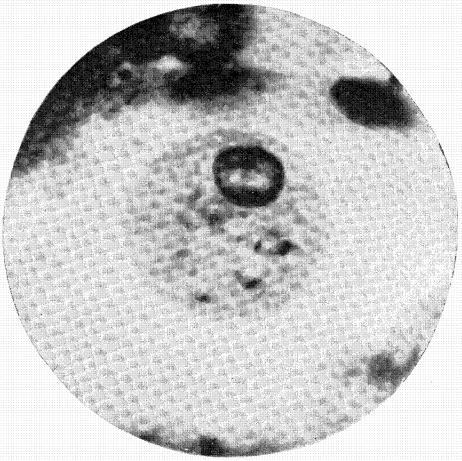

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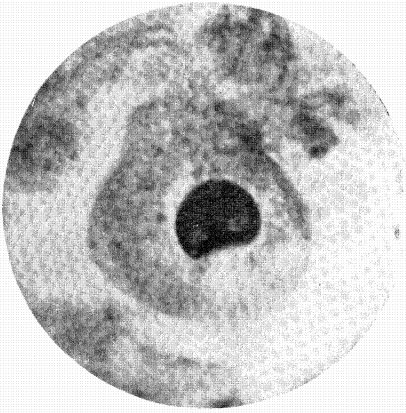

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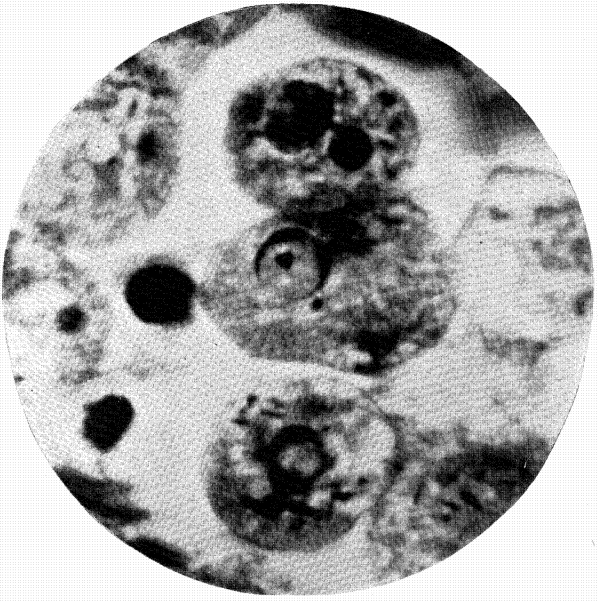
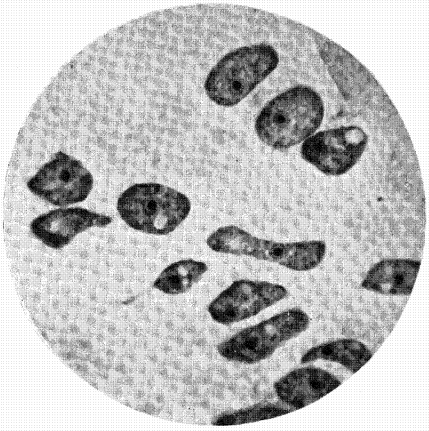


PLATE XXI.



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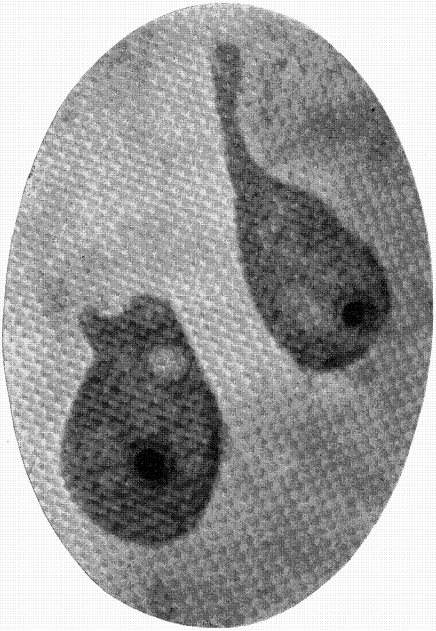

PLATE XXIII.

PREVIOUS PUBLICATIONS OF THE BUREAU OF GOVERNMENT LABORATORIES.

(Continued from second page of cover.)

Supply. By Charles L. Bliss, M. S. *Serum Laboratory:* V. Frambœsia: Its Occurence in Natives of the Philippine Islands. By Paul G. Woolley, M. D.

No. 21, 1904, Biological Laboratory.—Some Questions Relating to the Virulence of Micro-Organisms with Particular Reference to Their Immunizing Powers. By Richard P. Strong, M. D.

No. 22, 1904, Bureau of Government Laboratories.—I. A Description of the New Buildings of the Bureau of Government Laboratories. By Paul C. Freer. M. D., Ph. D. II. A Catalogue of the Library of the Bureau of Government Laboratories: By Mary Polk, Librarian.

No. 23, 1904, Biological Laboratory.—Plague: Bacteriology, Morbid Anatomy, and Histopathology (Including a Consideration of Insects as Plague Carriers). By Maximilian Herzog, M. D.

No. 24, 1904, Biological Laboratory.—Glanders: Its Diagnosis and Prevention, Together with a Report on Two Cases of Human Glanders Occurring in Manila and some Notes on the Bacteriology and Polymorphism of Bacterium Mallei. By William B. Wherry, M. D.

No. 25, 1904.[1]—Birds from the Islands of Romblon, Sibuyan, and Cresta de Gallo. By Richard C. McGregor.

No. 26, 1904, Biological Laboratory.—The Clinical and Pathological Significance of Balantidium Coli. By Richard P. Strong, M. D.

No. 27, 1904.—A Review of the Indentification of the Species Described in Blanco's Flora de Filipinas. By Elmer D. Merrill, Botanist.

No. 28, 1904.—II. The Polypodiaceae of the Philippine Islands. II. Edible Philippine Fungi. By Edward B. Copeland, Ph. D.

No. 29, 1904.—I. New or Noteworthy Philippine Plants, III. II. The Source of Manila Elemi. III. The Constituent Species of a Typical Philippine Hill Forest. By Elmer D. Merrill.

No. 30, 1905, Chemical Laboratory.—I. Autocatalytic Decomposition of Silver Oxide. II. Hydration in Solution. By Gilbert N. Lewis, Ph. D.

No. *31, 1905, Biological Laboratory.*—I. Notes on a Case of Haematochyluria, together with Some Observations on the Morphology of the Embryo Nematode, Filaria Nocturna. By William B. Wherry, M. D., and John R. McDill, M. D. II. A Search Into the Nitrate and Nitrite Content of Witte's "Peptone" with Special Reference to Its Influence on the Demonstration of the Indol and Cholera-Red Reaction. By William B. Wherry, M. D.

Publications of the Bureau are given out in serial number pertaining to the entire Bureau. Publications which may be desired can be obtained by applying to the Librarian of the Bureau of Government Laboratories, Manila, P. I., or to the Superintendent of Government Laboratories, Manila, P. I.

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[1]The first four bulletins in the ornithological series were published by the Ethnological Survey under the title "Bulletins of the Philippine Museum." Future ornithological publications of the Government will appear as publications of the Bureau of Government Laboratories.